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Voluntary Drinking of Ethanol by the Rat: Biogenic Amines and Possible Underlying Mechanism

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MESSIHA, F. S. *Voluntary drinking of ethanol by the rat: Biogenic amines and possible underlying mechanism.* PHARMAC. BIOCHEM. BEHAV. 9(3)379–384, 1978.—The present study evaluates the possible relationship between certain biogenic amine metabolites-produced changes in voluntary drinking of ethyl alcohol (ET) solution by the rat and their in vivo effects on the enzymes primarily involved in the hepatic metabolism of ET, i.e., liver alcohol-(L-ADH) and aldehyde dehydrogenase (L-ALDH). In experiments on voluntary intake of ET solution by the rat, compounds selected were injected, 0.5 mM/kg, IP. Administration of vanillylmandelic acid (VMA) and 5-hydroxyindoleacetic acid (5HIAA) and homovanillic acid (HVA) markedly reduced ET drinking. Similar significant effects were seen after administration of the neutral metabolites of the biogenic amines tested, after injection of metanephrine or 3-methoxy-4-hydroxyphenylpyruvic acid. Threodihydroxyphenylserine but not L-dopa reduced ET intake by the rat. Treatment with peripheral decarboxylase inhibitors, i.e., carbidopa, 50 mg/kg, IP, significantly reduced ET drinking as contrasted with nonsignificant decline in ET consumption following benserazide, 500 mg/kg, IP. In the biochemical study, short-term administration of the compounds selected produced varied effects on L-ADH and L-ALDH. It is suggested that alteration of hepatic ADH by the compounds tested might account for the observed reduced ET drinking, thereby indicating the contribution of peripheral sources rather than central factors in mediating the behavioral effects studied.

Biogenic amine metabolites Voluntary intake of ethanol Liver alcohol dehydrogenase
Liver aldehyde dehydrogenase Peripheral decarboxylase inhibitors Structure activity relationship

THE initial finding [32] of the rat's preference to drink diluted ethyl alcohol (ET) solution over water has provided an apparent simple animal model analogous to the compulsive drinking behavior seen in alcoholic patients. Like many animal models, the self-selection of ET solution over water by the rat has various shortcomings. However, it remains one of the most used behavioral performance technique in this regard. Furthermore, studying the efficacy of novel therapeutic agents on voluntary intake of ET may facilitate delineating the mechanism(s) underlying the urge for continued ET drinking. In view of the implication of some monoamines in certain aspects of ET-produced responses in [1, 4, 6, 9, 13–15, 19, 34, 35, 37] rodents, i.e., dependence on and preference to ET drinking, it is of interest to evaluate the effect of L-dopa, the dopamine precursor which penetrates the brain, and some of its major metabolites on voluntary intake of ET by the rat. The in vivo effects of some of these compounds on hepatic enzymes involved in the major metabolism of ET and acetaldehyde were studied to elucidate their role in the animal's behavioral response to ET drinking as modified by the agents selected.

METHOD

Behavioral Studies

Adult male Sprague-Dawley rats, 60–80 day old, were obtained from Holzman Farm, Madison, Wisconsin. The animals were housed individually for at least 14 days prior to experiments. The rats had access to Purina laboratory chow pellet food and to 5% (w/w) ET solution, prepared from 95% ET, as the only drinking fluid for an initial 14 day habituation period. This was followed by a 21-day period of free choice between water and the 5% ET solution. The two-fluid, three-bottle choice method has been used to prevent rats from selecting a fluid based on a position preference [28]. A drinking bottle of water, a bottle of 5% (w/w) ET solution and an empty bottle were mounted on each cage. Each day at 10:30 a.m. the amounts of fluids consumed during the preceeding 24 hr period were recorded. The bottles were refilled with the corresponding fluids, their weight recorded and put back on the cages and their positions rotated randomly from day to day. The amounts of food consumed and the body weight of the animals were measured and recorded every 24 hr and 48 hr, respectively. A set of two bottles, one for 5% ET solution and the other for water, were assigned to an empty cage and served to correct for fluid spillage and evaporation.

Animals preferring at least 60% of their daily consumption of drinking fluid as the 5% ET solution during a 3 week period following habituation were used for the study. A total of 182 rats were initially used over a 2-year period to obtain 98 animals preferring ET over water as the drinking

fluid. The results are expressed as percent changes occurring in food and fluids intake for the 24 hr period following the injection of the drugs tested from their respective mean pretreatment value referred to as base line, obtained by averaging the corresponding values for the 4-day period preceeding drug challenge. A *t*-test for correlated means was used for the statistical analysis.

Biochemical Studies

In a second series of experiments, the compounds studied in the behavioral experiments were administered once daily for 7 consecutive days to separate group of rats. The control animals received saline injections. The animals were then sacrificed by decapitation 16–18 hr after the final injection of the drugs or saline to avoid drug-induced hypothermia, if any. Their livers were quickly removed, weighed and individually homogenized in ice-cold 0.1 M KCl solution to obtain 15% (w/w) homogenates. The liver homogenates were subject to 22,000 × g centrifugation for 90 min. The resulting supernatans were assayed for alcohol dehydrogenase (L-ADH), (NAD, EC: 1.1.1.1), and aldehyde dehydrogenase (L-ALDH), (NAD, EC: 1.2.1.3) by the respective methods of Blair and Vallee [8] and Blair and Bodly [7] at pH 9.6 and 30°C. Protein determinations were made according to the biuret procedure.

All compounds were dissolved in saline and injected intraperitoneally (IP) in equimolar dose, 0.5 mM/kg, IP, unless otherwise indicated. These were as follows: L-3,4-dihidroxyphenylalanin ethylester (D), 5-hydroxy-tryptophan (5HTP), DL-threodihydroxyphenyl-serine (DOPS), dopamine (DA), 3-O-methyldopa (3OMD),3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxy-tryptamine (3MT), normetanephrine (NM), metanephrine (M), homovanillic acid (HVA) vanillylmandelic acid (VMA), 5-hydroxyindoleacetic acid (5HIAA), 3,methoxy-4-hydroxy-phenylethanol (MOPT), 3,4-dihydroxy-phenylglycol (DOPG), 3-methoxy-4-hydroxyphenylglycol (MHPG), 5-hydroxy-tryptophol (5HTOH), 3-methoxy-4-hydroxyphenylpyruvic acid (PP), 3-methoxy-4-hydroxyphenyllactic acid (PL), Benserazide, N-(DL-Seryl), N-(2,3,4-trihydroxybenzyl)-hydrazyl (BENS), 500 mg/kg, IP, Carbidopa, L- -methyl-β-(3,4,dihydroxyphenyl), propionic acid (CD), 50 mg/kg, and p-chlorophenylalanine (PCPA).

The results are expressed as percent changes in specific activity, nMol/min/mg protein, from the corresponding controls. The statistical significance of the results was evaluated by two-tailed student *t* test for independent means.

RESULTS

Figure 1 shows the effects of DOPS, L-dopa and their metabolites selected on voluntary intake of ET solution by the rat. All compounds were injected 0.5 mM/kg, IP once. The results are expressed as percent changes occurring in fluids and food intake during the 24 hr following drug administration from the mean 4 days value preceeding drug injection. Administration of D did not influence ET drinking compared to 26% ($p<0.05$) and 72% ($p<0.001$) reductions occurring subsequent to DOPS and M, respectively. Metanephrine-treated rats showed the most marked reduction in their food and fluid intake from their mean base line values. By contrast injections of 3MT, or NM, failed to alter ET intake by the rat. HVA and 5-HIAA, the respective major acidic end products of DA and serotonin metabolism in the peripheral tissues, exerted a respective 48% ($p<0.01$) and 61% ($p<0.01$) reduction in ET drinking concomitant with a compensatory significant ($p<0.01$) rise in water intake. Injection of VMA, the major acidic metabolite of NE, moderately decreased ET intake, by approximately 32% ($p<0.05$). Administration of equal mM doses of the neutral metabolites of the biogenic amines tested resulted in 25% to 33% ($p<0.05$) reduction in voluntary drinking of ET from predrug treatment with a greater reduction, 51% ($p<0.05$), occurring subsequent to treatment with 5HTOH, the major neutral metabolite of serotonin. Injection of PP was associated with decreased, 35% ($p<0.01$), ET intake and a rise in water drinking ($p<0.001$). Administration of PL, or saline exerted little effects on fluid and food consumption from pretreatment levels.

Figure 2 shows the effect of peripherally acting inhibitors of aromatic L-amino acids decarboxylase on voluntary intake of 5% ET solution by the rat. Injection of BENS, 500 mg/kg, IP, reduced ET drinking by approximately 40% for the 24 hr thereafter. This decrease occurred in 5 of the 6 rats tested and was not statistically different from mean pretreatment value. Furthermore, there was a decreased food consumption for two days period following injection of BENS. Administration of CD, 50 mg/kg, produced a greater and significant ($p<0.02$) decline in the 24 hr intake of ET without concomitant changes in food or water consumption. All animals maintained their normal growth rate subsequent to drug administration.

Table 1 shows the effects of biogenic amines and metabolites selected on hepatic ADH and ALDH activities. Rats were injected saline or one of these compounds, 0.5 mM/kg/day for 7 consecutive days, and sacrificed 16 hr after final treatment. The results are expressed as percent changes of specific activities from the corresponding saline-treated controls (=100%). Short-term administration of MHPG, the major neutral metabolite of NE, or PL inhibited L-ADH ($p<0.05$) compared to saline-treated controls. This is contrasted with induction of rat L-ADH by VMA and 5HTOH ($p<0.001$) the respective acidic and neutral metabolite of NE and serotonin. Cytoplasmic L-ALDH was not markedly altered by any of the compounds listed in Table 1 and the decrease in L-ALDH activity after injection of MHPG or PL was not statistically significant ($p<0.1$).

DISCUSSION

Hypotheses have emerged relating biogenic amines involvements in the mechanism(s) underlying voluntary selection of diluted ET solution over water as the drinking fluid by the rat [3, 16, 17, 20–22, 29–31]. This is partially due to the implicit assumption that most agents producing addictive states and physical dependence in man, i.e., ET, are probably associated with continued dynamic interaction between the addictive agent and/or metabolites with certain target systems containing suspected putative neurotransmitters, i.e., serotonin, DA or NE, which can evoke behavioral changes as a consequence of alteration in their biochemical function by pharmacologic interventions. Studies performed in this context have utilized different routes of drug administration and the resulting patterns of behavioral response to ET drinking are usually inferred to central origin. However, few studies, if any, have reported the aversive effects of drug treatment in reducing ET intake as related to changes in food consumption and body weight or considered the possible formation of intermediate metabolites of the exogenously

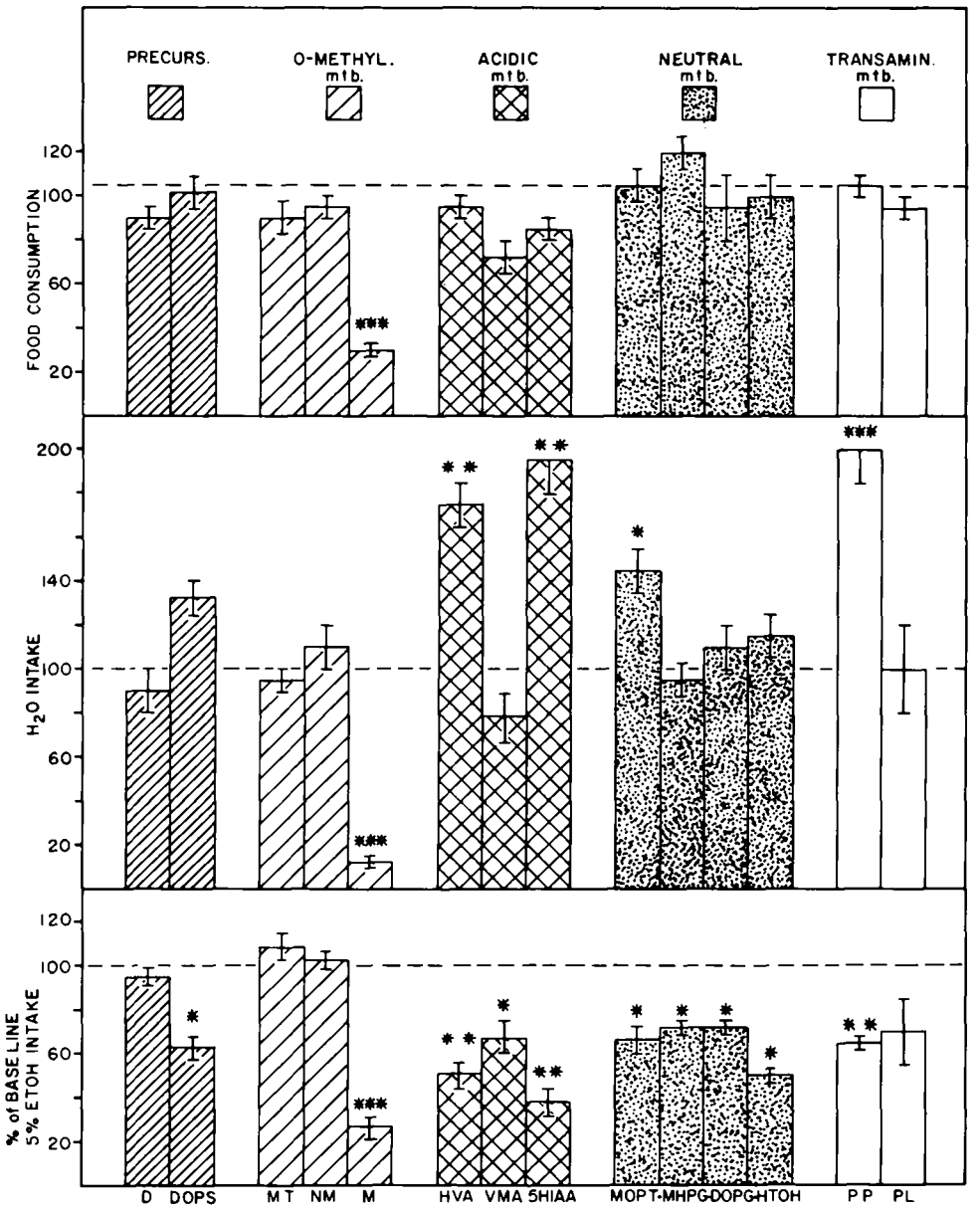
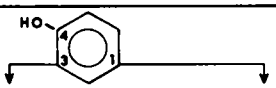
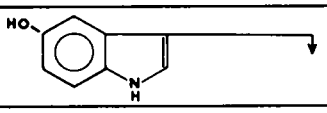
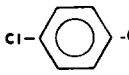


FIG. 1. The effects of L-dopa (D) and threodihydroxyphenylserine (DOPS) and some metabolites on voluntary intake of 5% (w/w) ethanol (ET) solution by the rat. L-dopa, O-methylated (O-methyl.), acidic-, neutral- and transaminated-(Transam.) metabolites (mtb) and DOPS were injected in equimilimolar doses, 0.5 mM/kg, IP. Alterations in fluid intake and food consumption during the 24 hr of drug injections are expressed as percent changes from corresponding mean base line values, obtained by averaging daily results deriving from the immediate 4-day period preceeding drug treatments. Values are means ± SE for 5–7 independent experiments for each drug trial. A total of 98 rats were used. For the abbreviation used see text. Significance was evaluated by t test for correlated means. $^{***}p<0.001$; $^{**}p<0.01$; $^{*}p<0.05$.

administered monoamines (see Fig. 3) which may possess different properties than their precursors. In the present study, injection of M reduced ET drinking by the rat concomitant with marked toxic manifestations as evident by reduction in fluid and food consumptions. Furthermore, the use of inhibitors of peripheral D decarboxylase, resulted in decreased voluntary intake of ET. This suggests that the effect observed is most likely of peripheral origin since both drugs have been shown to inhibit L-ALDH in vivo [23,25], which is possibly due to the formation of hydrogen peroxides and free radicals known to occur from the demethylation of certain aromatic hydrazine compounds [2,36]. It is well

TABLE 1

THE EFFECT OF SHORT-TERM ADMINISTRATION OF CERTAIN MONOAMINES AND METABOLITES ON SPECIFIC ACTIVITY OF RAT LIVER ALCOHOL-(L-ADH) AND ALDEHYDE DEHYDROGENASE (L-ALDH).

Compounds Metab.	Abrv.			% of Saline Controls L-ADH	L-ALDH
Precursors					
	D	OH	$-CH_2-CH-NH_2$ COOH	101.0 ± 7.6	85.1 ± 10.0
	3OMD	OCH_3	$-CH_2-CH-NH_2$ COOH	104.8 ± 8.2	87.5 ± 13.6
	DOPS	OH	$-CHOH-CHNH_2$ COOH	104.8 ± 8.2	115.1 ± 9.6
O-Methylated Amines					
	3MT	OCH_3	$-CH_2-CH_2-NH_2$	96.7 ± 11.6	87.5 ± 8.8
	NM	OCH_3	$-CHOH-CH_2NH_2$	11.4 ± 15.8	100.2 ± 9.6
	M	OCH_3	$-CHOH-CH_2NH\ CH_3$	101.0 ± 14.2	119.7 ± 15.4
Neutral					
	MOPT	OCH_3	$-CH_2-CH_2OH$	100.8 ± 8.8	100.2 ± 1.5
	MHPG	OCH_3	$-CHOH-CH_2OH$	84.8 ± 3.8‡	83.3 ± 3.9§
Acidic					
	HVA	OCH_3	$-CH_2-$ COOH	111.0 ± 5.9	93.9 ± 9.5
	VMA	OCH_3	-CHOH- COOH	118.4 ± 5.9†	77.8 ± 15.5
Transaminated					
	PL	OCH_3	$-CH_2-CHOH-$ COOH	71.7 ± 9.6	85 ± 8.7
	PP	OCH_3	$-CH_2-CO-$ COOH	116.5 ± 15.5	71.7 ± 9.6§
					
Precurs.	5HTP		$-CH_2-CH-NH_2$ COOH	113.7 ± 5.8	91.8 ± 9.3
Neutral	5HTOH		$-CH_2-CH_2OH$	146 ± 13.0*	100.1 ± 18.5
Acidic	5HIAA		$-CH_2-$ COOH	95.8 ± 3.3	102 ± 2.6
Inhibitor	PCPA		$-CH_2-CH-NH_2$ COOH	110.0 ± 2.0	83.7 ± 12.2

Values are means ± SE of % changes in specific activity of rat liver alcohol dehydrogenase and aldehyde dehydrogenase from saline treated controls (=100%). Compounds listed were injected in equimilimolar concentration 0.5 mM/kg, IP, once daily for 7 consecutive days and the animals were sacrificed 16–18 hr thereafter. Each value derives from 6–8 independent experiments. A total of 136 rats were used for the saline and drug-treatments.

*$p<0.001$.

†$p<0.01$.

‡$p<0.05$.

§not significant ($p<0.1$)

known that 85–95% of the amounts of ET ingested is primarily metabolized by hepatic ADH, the probable rate limiting step in the metabolism of ET. Thus, a systematic approach to the clarification of drug effects, in the presently used behavioral performance test, may have to include studies on the effects of the drugs tested on hepatic ADH and ALDH. The present results show that most compounds tested produced varied effects on voluntary drinking of ET solution with some altering specific activity of L-ADH. For example, inhibition of L-ADH, i.e., by MHPG or PL, may result in increased ET blood levels and subsequent reduction in ET intake. Similarly, inhibition of ALDH, i.e., by PP, may result in a buildup of ET-derived acetaldehyde and augmentation of ET toxicity which is then manifested by reduced ET drinking by the rat. The same effect may occur in incidences where L-ADH is induced, i.e., by 5HTOH or VMA, which may cause a rise in blood ET levels. Moreover, induction of any of these enzymes will result in accelerated utilization of NAD and subsequently increased NADH:NAD which can contribute to reduced ET intake [18,37]. Thus, changes in L-ADH, L-ALDH and NAD:NADH may all conceivably alter ET intake without directly being related to ET actions in the brain. Similarly, the lack of effect of the D-dose given on voluntary ET intake indicate that previously reported decrease in ET drinking by D is probably dose-dependent and is partially due to the combined cardiovascular effects of ET and to the large D-dose given [29]. A further mechanism which might have contributed to the observed reduction in ET drinking by the acidic- and neutral metabolites studied may reside in ET-produced shift in the major metabolism of the biogenic amines from their predominant oxidative pathway to the reductive route of the metabolism [11,12]. Ac-

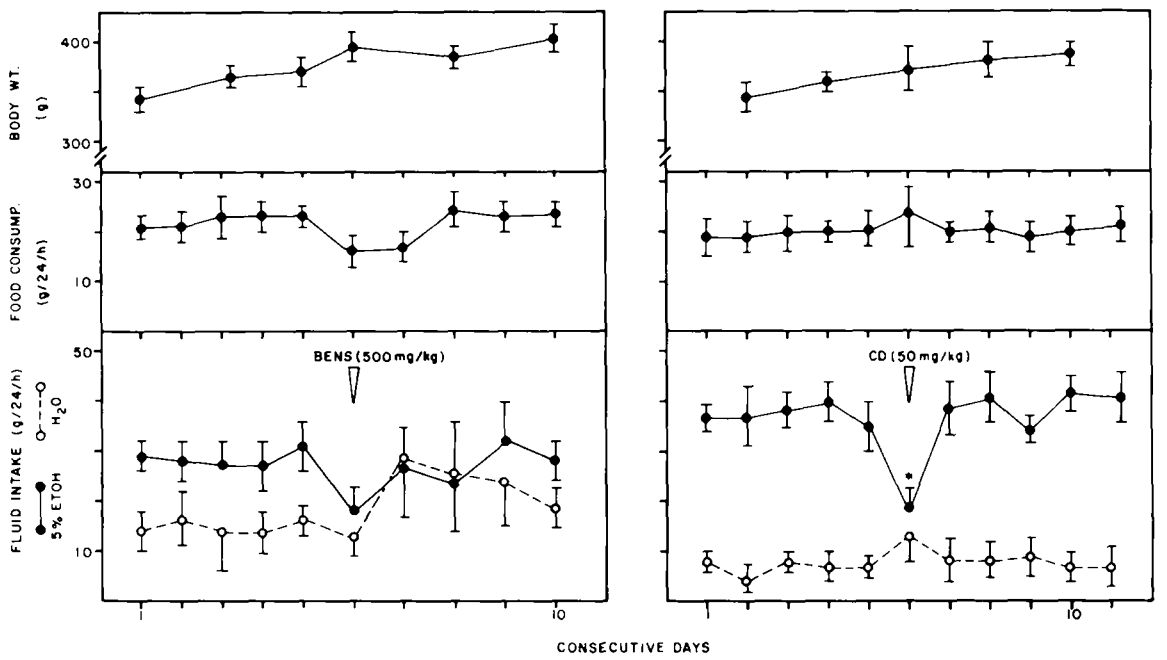


FIG. 2. The effect of benserazide (BENS) and carbidopa (CD) on voluntary drinking of ethanol (ET) solution by the rat. Arrows indicate time of BENS, 500 mg/kg, and CD, 50 mg/kg, injection. Lower panels show 24 hr intake of 5% ET (w/w) ●——● and water ○. . . .○ (g/24 hr), followed by amounts of food consumed (g/24 hr) and changes in body weight (g) at the times indicated. Values are means ± SE of 6 animals for each drug trial. *$p<0.02$.

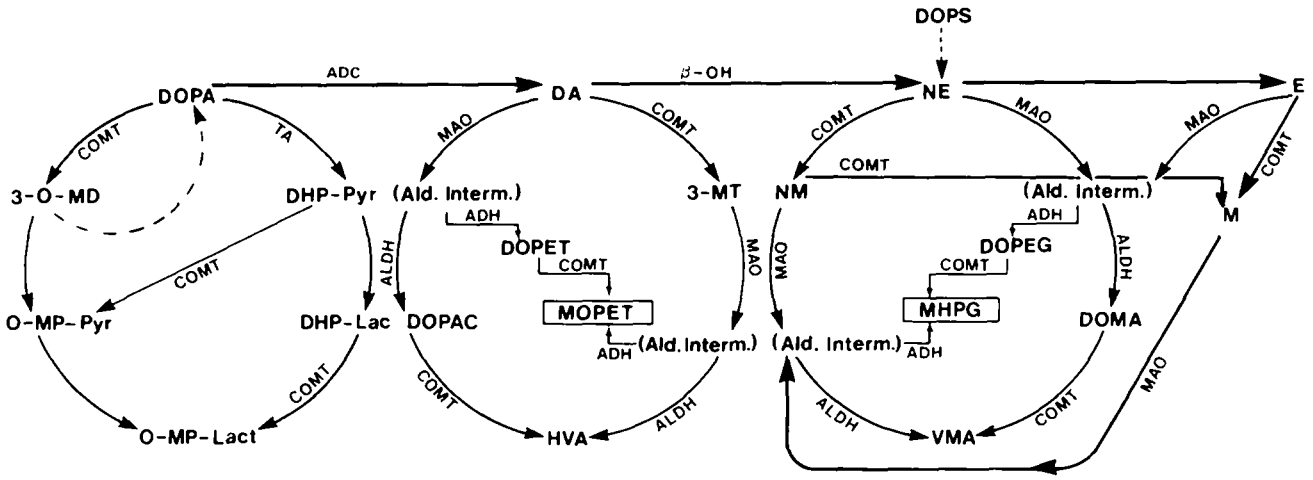


FIG. 3. The major and alternative metabolic pathway of L-dopa in the mammalian tissue. The major metabolism of D usually proceeds by decarboxylation of D to DA and the subsequent formation of the O-methylated and oxidative deaminated intermediates prior to the formation of the major acidic metabolites DOPAC and HVA. A small portion of exogenously administered D is converted to VMA through norepinephrine (NE) pathway. Inhibition of D decarboxylase by drugs preferentially acting on extracerebral aromatic L-amino acid decarboxylase (ACD), i.e., BENS or CD, divert the major metabolism of D to the transamination route and increase the formation of 3-OMD [5] prior to its conversion to HAV through pyruvic- and lactic acid pathway as has been suggested [26] and experimentally proven [33]. For abbreviations see text. Other abbreviations are: dopa decarboxylase (DC), dopamine β-hydroxylase (β-OH), monoamine oxidase (MAO), Catechol O-methyl transferase (COMT), Transaminase (TA), Dihydroxyphenylpyruvic acid (DHP-Pyr), dihydroxyphenyllactic acid (DHP-Lac), 3-methoxy-4-hydroxyphenyl-pyruvic acid (OMP-Pyr) and 3-O-methyl 4, hydroxyphenyllactic acid (O-MP-Lact).

cordingly, increased formation of the neutral metabolites, i.e., primary and secondary alcohol derivatives, may compete with the substrate ET for the enzyme ADH in the liver. Furthermore, it should be noted that compounds with indole moity, i.e., 5HTOH and 5-HIAA, produced the most profound reduction on ET intake suggesting the importance of the indole nucleus in this behavioral test.

In conclusion, the present results strongly suggest that interpretation of central drug effect in experiments on voluntary intake of ET should consider the effect of the drug on hepatic L-ADH and ALDH prior to the advancements of behavioral hypotheses related to drug action and ET-drug interactions.

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