3-Methylbutanal metabolism in the adult rat

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Summary
1. 3-Methylbutanal is a normal constituent of human plasma and is elevated in patients with hepatic encephalopathy. Therefore these studies examined the possible source and site of synthesis of 3-methylbutanal and its effect on the central nervous system of adult rats.

2. 3-Methylbutanal was found to be a normal constituent of rat plasma and increased two to five times when leucine comprised 5% of the diet. Neomycin in the diet prevented the leucine-induced rise in plasma 3-methylbutanal. When this was injected at a dose of 120 mg/kg abnormal EEG patterns and sleep-like behaviour occurred, whereas smaller amounts (30 mg/kg) increased brain serotonin concentrations.

3. 3-Methylbutanal is a normal component of rat plasma: it may be derived in part from colonic bacterial breakdown of leucine and may influence central nervous system function. A possible relationship of 3-methylbutanal to the pathogenesis of hepatic encephalopathy is suggested.

Key words: hepatic encephalopathy, leucine, synthesis.

Introduction
Hepatic encephalopathy is a complex neuropsychiatric syndrome which can complicate all forms of liver disease and may be defined as a disturbance in cerebral function due to the combination of advanced liver disease and portal systemic shunting. Present concepts of the pathogenesis of hepatic encephalopathy emphasize that altered cerebral function may be caused by various metabolic products of gut origin which are normally taken up from portal vein blood by the intact liver, but, in the presence of liver disease or portosystemic shunts, pass into the systemic circulation. The exact cerebral toxin(s) causing hepatic encephalopathy are uncertain.

In recent years, one focus of research has been on toxic substances produced from the metabolism of protein by intestinal bacteria. Bacterial catabolism of amino acids may produce many low-molecular-weight substances including volatile aldehydes and ketones. In a recent study patients with signs of hepatic encephalopathy were found to have significantly elevated plasma levels of 3-methylbutanal, a possible derivative of leucine catabolism [1]. Accordingly, we have examined, in the adult rat, (a) the effect of increasing dietary leucine on 3-methylbutanal production; (b) a possible site of 3-methylbutanal synthesis; (c) the effect of 3-methylbutanal on brain function and neurotransmitter metabolism.

Methods
The first series of experiments was designed to obtain data on normal plasma levels of 3-methylbutanal in rat plasma and the effect of age, dietary leucine and neomycin intake on these levels. The objective of the second group of experiments was to provide evidence that 3-methylbutanal could alter central nervous system function, as measured by electroencephalogram (EEG), and neurotransmitter metabolism, as measured by serotonin and 5-hydroxyindoleacetic acid concentrations.

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Male Wistar rats (Canadian Breeding Laboratories, Montreal, Quebec, Canada) were housed in individual wire meshed galvanized cages in a room with controlled lighting (08:00–20:00 hours daily) and maintained at approximately 23°C. Fresh food and water were available ad lib. During the first week in the animal quarters the rats were permitted free access to commercial food pellets (Purina rat chow, Ralston Purina of Canada, Woodstock, Ontario, Canada) and water before introduction of the experimental protocols.

The purified test diets given contained 20% casein [2] to which either 5% leucine by weight or 0.1% neomycin was added. Food intake and body weight were recorded of rats given these test diets for either 1 or 2 weeks. In the injection studies the rats were injected intraperitoneally with either 50 μl of sodium chloride solution (0.9%, 150 mmol/l: saline) or 30, 60 or 120 mg of 3-methylbutanal/kg body weight (Aldrich Chemicals Co, Inc., Milwaukee, WI, U.S.A.) by means of a 50 or 100μl Hamilton syringe with a ½ inch 25-gauge Yale needle.

EEG recordings were performed on freely moving animals with chronically implanted electrodes by conventional stereotaxic techniques [3].

In preparation for EEG recordings, stainless steel bipolar recording electrodes (no. M5303/1, Plastics Products Co., Roanoke, VA, U.S.A.) were implanted 2.5 mm anterior to lambda and 3.0 mm lateral to the midline of the visual cortex to record the electrical activity of the raphe nuclei [4].

The rats were killed by guillotine between 09:00 and 11:30 hours in all experiments and brain tissue and plasma collected for amino acid analysis as described previously [2]. To obtain intestinal material for bacteriological culturing the caecal contents were removed.

Gas chromatographic analyses for plasma 3-methylbutanal determination were carried out by a method adapted from Goldberg et al. [1] with a Shimadzu GC-Mini 1 (Shimadzu Seisakusho, Ltd, Kyoto, Japan) equipped to handle capillary columns and dual flame-ionization detectors. The method is based on the direct injection, by a low-volume injector, of 2 μl of the plasma sample through a glass liner packed with glass beads (60 mesh) on to a wall-coated open tubular glass capillary column. The carrier gas was nitrogen, at a flow rate of 10 cm/s. The injector temperature was 250°C. Column temperature was 350°C. The column was kept isothermal for 5 min, then programmed at 2°C min to 180°C. The concentration of 3-methylbutanal in the sample was computed relative to the internal standard, butan-1-ol, added to the sample to give a concentration of 5 p.p.m. (v/v).

For the whole brain was divided mid-sagittally, one-half to be analysed for tryptophan (Trp), serotonin and 5-hydroxyindole-acetic acid according to the method of Curzon & Green [5] as revised by Weinberger et al. [6] but with one modification. More complete extraction of 5-hydroxyindole-acetic acid was obtained by using 1.0 ml of NaHCO₃ (0.033 mol/l) instead of phosphate buffer (0.5 mol/l) at pH 7.0 [7]. An equal number of right and left hemispheres was used for assay.

Caecal specimens were weighed and homogenized in a nutrient broth consisting of chopped meat carbohydrate sealed anaerobically (Carr-Scarborough Microbiological Inc., Decator, GA, U.S.A.), to culture and estimate urease-positive bacteria. Serial logarithmic dilutions of the homogenate were made in duplicate up to 10⁻¹² and all inoculated media were incubated at 37°C for 24 h. One of each of the duplicate broth tubes were injected with urea solution to a concentration of 2%. All cultures were incubated at 37°C for 6 h. Equal volumes of each tube were spotted with a drop of phenol red solution (0.02%) with a change of phenol red to blue indicating the presence of urease-positive bacteria.

Correlation analysis and Student’s t-test were conducted as appropriate [8]. A multiple-comparison test, with an estimate of the pooled variance, was also used to analyse the data.

**Results**

Plasma of both young and adult rats given casein diets contained 3-methylbutanal (Table 1). Leucine diets (5%, w/w) caused the levels to be increased 4.7 and 2.1 times in young and adult rats respectively. In the older rats, where individual plasma leucine data were available, the plasma leucine and 3-methylbutanal levels were significantly correlated (r = 0.50, P < 0.05).

Neomycin in the diet prevented the increase in plasma 3-methylbutanal caused by the high-leucine diet (Table 2) and decreased urease-positive facultative anaerobe concentrations in the caecum. The mean level of bacteria in control animals was 10⁸ (range 10⁷⁻¹⁰¹⁰) per g wet weight, whereas the average number of bacteria in neomycin-treated animals was 10⁴ (range 10⁶⁻¹⁰⁸) (P < 0.05). No significant difference was seen in the average weights of caecal contents between neomycin-treated animals (2.1 ± 0.7 g, n = 12) and those of control animals (2.2 ± 0.2 g, n = 12).
When 3-methylbutanal was injected at increasing levels into two 200 g rats in doses of 4, 20, 40, 80 and 120 mg/kg body weight the 120 mg/kg dose was found to result in reproducible changes in the EEG from a resting to a sleep pattern within 2–10 min. Wave frequency diminished and the amplitude increased as compared with baseline recordings (Fig. 1) and the EEG indicated the presence of spindle-like waves which are found in slow-wave sleep [9]. Concomitant with these EEG alterations were overt signs of drowsiness or sleep, with the lower doses having a similar but less marked effect. 3-Methylbutanal at 800 mg/kg was a lethal dose.

In a subsequent experiment 3-methylbutanal was injected at 30, 60 or 120 mg/kg 9 min before the rats were killed. Plasma 3-methylbutanal reflected the injections (Table 3). All dose levels

### Table 1. Plasma leucine and 3-methylbutanal levels in young and adult rats given high-leucine diets

<table>
<thead>
<tr>
<th></th>
<th>Weight gain (g)</th>
<th>Food intake (g)</th>
<th>3-Methylbutanal (mg/l)</th>
<th>Leucine (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Young rats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein control</td>
<td>50.2 ± 2.0</td>
<td>130.2 ± 3.8</td>
<td>0.293 ± 0.050</td>
<td>294‡</td>
</tr>
<tr>
<td>High-leucine</td>
<td>47.8 ± 1.4</td>
<td>124.5 ± 2.5</td>
<td>1.377 ± 0.343§</td>
<td>430</td>
</tr>
<tr>
<td><strong>Adult rats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein control</td>
<td>21.5 ± 3.2</td>
<td>249.5 ± 6.9</td>
<td>0.346 ± 2.60</td>
<td>189 ± 11</td>
</tr>
<tr>
<td>High-leucine</td>
<td>22.5 ± 2.1</td>
<td>252.0 ± 5.1</td>
<td>0.711 ± 0.97§</td>
<td>289 ± 28§</td>
</tr>
</tbody>
</table>

* Initial weight 205 ± 0.9 g (n = 10).
† Initial weight 416 ± 5.6 g (n = 8).
‡ Pooled analyses.
§ Significantly greater than control (P < 0.05).

### Table 2. Plasma 3-methylbutanal levels in rats given casein and high-leucine diets with neomycin

<table>
<thead>
<tr>
<th></th>
<th>Weight gain (g)</th>
<th>Food intake (g)</th>
<th>3-Methylbutanal (mg/l)</th>
<th>Leucine (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Casein control</strong></td>
<td>99.3 ± 9.0†</td>
<td>274.4 ± 6.8</td>
<td>0.464 ± 0.097</td>
<td>264 ± 41</td>
</tr>
<tr>
<td>+ Neomycin</td>
<td>103.9 ± 7.8</td>
<td>266.0 ± 8.7</td>
<td>0.500 ± 0.137</td>
<td>262†</td>
</tr>
<tr>
<td><strong>High-leucine</strong></td>
<td>106.5 ± 6.0</td>
<td>270.7 ± 6.2</td>
<td>1.142 ± 0.251‡</td>
<td>372 ± 26</td>
</tr>
<tr>
<td>+ Neomycin</td>
<td>104.4 ± 4.6</td>
<td>270.4 ± 8.5</td>
<td>0.436 ± 0.068</td>
<td>360†</td>
</tr>
</tbody>
</table>

* Mean ± SEM (n = 8); initial body weight 209 ± 1.0 g.
† Pooled analyses.
‡ Significantly greater than all other groups (P < 0.05).

**FIG. 1.** Effect of 3-methylbutanal on the EEG of the conscious rat. (a) Cortical EEG recorded 30 s before injection of 3-methylbutanal. (b) EEG taken 10 min after administration of 120 mg of 3-methylbutanal/kg. (c) Cerebral electrical activity after administration of saline.
increased the plasma tryptophan (Trp)/neutral amino acid ratios. Brain 5-hydroxyindole-acetic acid was increased, but only in those animals receiving the lower dose of 30 mg/kg.

**Discussion**

The presence of 3-methylbutanal in the plasma of normal rats suggests that it is a normal circulatory substance and that it originates naturally from metabolic processes. The mean levels of 3-methylbutanal in normal rat plasma (0.46 ± 0.1 mg/l) correspond to levels found in normal man (0.58 ± 0.22 mg/l) [1] making comparisons of the higher levels after injection also of interest.

The increased plasma 3-methylbutanal levels observed with high-leucine diets and their correlation with plasma leucine levels suggest that plasma 3-methylbutanal is derived from leucine. However, the present study does not establish whether leucine is the only source of 3-methylbutanal and does not establish whether the appearance of 3-methylbutanal in plasma is entirely dependent on gut bacteria or produced endogenously from mammalian tissue. The finding that neomycin addition to the high-leucine diet prevented the rise in plasma levels of 3-methylbutanal (0.46 ± 0.1 mg/l) correspond to levels found in normal man (0.58 ± 0.22 mg/l) [1] making comparisons of the higher levels after injection also of interest.

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The increased brain 5-hydroxyindole-acetic acid levels after administration of 30 mg of methylbutanal/kg, but not with the two higher doses of 3-methylbutanal, is difficult to explain (Table 2), but may be because of effects similar to that of other short-chain aldehydes and alcohols. Acute administration of a single dose of ethanol has been shown to cause a biphasic effect on noradrenaline (11) and serotonin turnover in the brain, with early increases and subsequent decreases. Thus it is possible that the injection of the higher doses of 3-methylbutanal may have produced a similar biphasic effect on brain serotonin turnover such that increases in brain 5-hydroxyindole-acetic acid concentrations were missed.

**Acknowledgments**

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References


