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Some characteristics of the metabolism of β-phenylethylamine in rat isolated lung

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β-Phenylethylamine (PEN) is metabolized on a single passage through the pulmonary circulation of rats and rabbits (Bakhle & Youdim, 1976; Roth & Gillis, 1975) and the metabolism of this amine involves monoamine oxidase of the B type (MAO-B; Yang & Neff, 1973) in contrast to that of 5-hydroxytryptamine (5-HT) which involves MAO-A. Both these enzymes are intracellular and amine metabolism must therefore be preceded by transfer of substrate across the cell membrane. Either step, uptake or enzymic reaction, could be rate-limiting.

We have studied the kinetics of the metabolism of PEN and of 5-HT in isolated rat lungs, by measuring the amine metabolites over a range of concentrations. Amine labelled with ^{14}C was infused through isolated lungs, perfused with Krebs solution via the pulmonary artery, for 3 min and the effluent collected during, and after, the infusion for a total of 30 minutes. By this time the radioactivity in the effluent had fallen to background levels. Aliquots of the collected effluent were chromatographed on ion exchange columns (Amberlite CG-50; Southgate & Collins, 1969) and the radioactivity eluted from the columns with water (non-basic metabolites) measured by liquid scintillation methods.

For PEN over a concentration range of 0.1–150 μM, metabolism seems to be governed by a single rate-limiting step with an apparent K_m of 55 μM and V_{max} of 880 nmol $lung^{-1}$ 30 min^{-1}. In contrast, the metabolism of 5-HT over a similar range (0.1–50 μM) seems best described by two processes; one with K_m 2 μM and V_{max} 34 nmol and the other with a K_m value at least 25 times higher. The K_m values for metabolism in the perfused organ should be compared with the K_ms for 5-HT, 187 μM, and PEN, 20 μM, obtained with MAO isolated from liver (Houslay & Tipton, 1974).

Metabolism of PEN in lung is not inhibited by 5-HT (Bakhle & Youdim, 1976) and in these experiments, tryptamine and benzylamine (0.2 and 2.0 μM) did not inhibit the metabolism of ^{14}C-PEN (0.15 μM). Furthermore, the inactivation of noradrenaline (0.5–2 ng/ml) in isolated lungs, measured by bioassay, was not affected by PEN infused through the lung in concentrations up to 500 ng/ml. Tricyclic antidepressant drugs such as amitriptyline and imipramine inhibit uptake and hence metabolism of 5-HT and noradrenaline in lung (for refs. see Bakhle & Vane, 1974) but PEN metabolism was not inhibited by desmethylimipramine (10^{-5} M). However, in lungs from rats pretreated (3 h; 5 mg/kg i.p.) with deprenil, an inhibitor of MAO-B (Knoll & Magyar, 1972), metabolism of PEN was inhibited by 34%.

From these results, the rat lung has a greater capacity to metabolize PEN compared with 5-HT and the process by which PEN enters the cell may be different from those uptake systems already described for 5-HT and noradrenaline in the lung (Gillis, 1976).

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The metabolic fate of [^{14}C]-phenobarbitone in the rat and the effect of chronic administration and dose size

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The metabolic fate of phenobarbitone (5-ethyl-5-phenylbarbituric acid) has been studied in the rat as part of an examination of the biochemical basis of the tolerance which occurs to this drug. The effect of dose size and chronic administration on its metabolism has also been examined. [^{14}C]-Phenobarbitone, labelled in the 2-position of the barbituric acid ring (50 mg/kg; 10 μCi/animal), was injected intraperitoneally into female Wistar albino rats (body weight 180–200 g). Urine and faeces were collected for 8 days after administration and $^{14}CO_2$ in the expired air collected for 2 days. The excretion of ^{14}C was monitored by liquid scintillation counting and the metabolites present in the urine examined by thin layer chromatography followed by radiochromatogram scanning, reverse isotope dilution, gas-liquid chromatography and gas chromatography-mass spectrometry (g.c.-m.s.). Over the 8 days of the experiment, some 88% of the administered ^{14}C was recovered, with 79% in the urine, 0.14% as $^{14}CO_2$ in the expired air and 9% in the faeces. The bulk (75%) of the urinary ^{14}C was excreted in the first 3 days, with 43% appearing in the first 24 hour. After the 4th day, some 0.5–1% of the dose was excreted in the urine per day. Analysis of the 0–24 h urine by the techniques described revealed the presence of unchanged phenobarbitone (14% of dose), *p*-hydroxy-phenobarbitone, excreted free (16%) and as its glucuronide conjugate (9%) and an unknown acidic water-soluble metabolite (4%). The identities of these metabolites were confirmed by comparison of their g.c.-m.s. properties with standards, and the g.c.-m.s. of the unknown suggested that it was ethylphenyl-malonylurea, arising from the ring opening of phenobarbitone. The nature of the faecal radioactivity was not determined. Phenobarbitone and its metabolites were excreted at similar rates, and since the excretion half life of total ^{14}C was quite long (ca. 1.25 days) it would be expected that phenobarbitone and its metabolites would be accumulated when the drug was administered on a daily basis.

In other experiments, the excretion of [^{14}C]-phenobarbitone by bile-duct cannulated rats was examined. After the i.p. injection of 50 mg/kg (10 μCi/animal), 35% of the dose was excreted in the bile in 24 h with a further 18% in the urine. The excretion of ^{14}C in the bile proceeded at a fairly constant rate of about 1.5% of dose/h over the 24 h of the experiment. The metabolites present in bile were examined as above, and comprised phenobarbitone (5% of dose) *p*-hydroxyphenobarbitone glucuronide (21%) and the unknown metabolite (9%). No free *p*-hydroxyphenobarbitone was found in bile.

The possible variation in the metabolism of phenobarbitone with dose was also studied. Using the methods described, rats were dosed with 5 mg/kg and 100 mg/kg [^{14}C]-phenobarbitone by i.p. injection (10 μCi/animal), and the excreta analysed for ^{14}C and metabolites. With both these doses, the rate of excretion and nature of the metabolic products was essentially similar to the findings reported with a 50 mg/kg dose.

Analysis of the tissue distribution of ^{14}C 24 h after the i.p. injection of [^{14}C]-phenobarbitone (50 mg/kg; 20 μCi/animal) showed that the bulk of the radioactivity remaining unexcreted was associated with the muscle, fat and bones of the carcass (16%) and in the blood (red cells 3%/ml; plasma 1%/ml). Some 7% of the dose was associated with the vital organs, in the order liver > kidney > lung > heart > gut wall > brain, the brain containing 0.2% of dose.

In further experiments, rats were treated daily with phenobarbitone (50 mg/kg i.p.) (treated group) or the injection vehicle, 90% propane-1,2-diol (2 ml/kg i.p.) (control group) for up to 14 days. Groups of rats were taken after 4 days or 14 days chronic administration, injected with [^{14}C]-phenobarbitone (50 mg/kg; 10 μCi/animal) and their urine and faeces collected for 3 days. Analysis of ^{14}C and metabolites in the urine was performed as described. The excretion of ^{14}C in the urine and faeces of both treated and control