DEMONSTRATIONS

Separation and quantification of metabolites of radiolabelled histamine in brain

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As part of an investigation of the function of histamine in the central nervous system, we have studied the metabolism *in vivo* and *in vitro* of [^{14}C]-histamine and its major brain metabolite [^{14}C]-*tele*methylhistamine (see review by Schwartz, 1977), in the rat. We describe here a method which is suitable for separation and quantification of radiolabelled histamine, imidazoleacetic acid, *tele*methylhistamine, and *tele*methylimidazoleacetic acid (nomenclature of Black & Ganellin, 1974).

Investigation of metabolism *in vivo* of [^{14}C]-histamine and [^{14}C]-methylhistamine was carried out as follows. The labelled substance in artificial cerebrospinal fluid (10 µl) was injected into the right lateral ventricle via a cannula guide (Hayden, Johnson & Maickel, 1966). The rat was killed by cervical dislocation, the brain rapidly removed and washed in saline, and homogenized in 0.6 M perchloric acid (5 ml) containing 20 µg each of unlabelled carrier substances. The homogenate was centrifuged at 20000 *g* for 10 min; the pellet was washed twice with water and all three supernatants were combined. The combined supernatant was brought to approx. pH 6 with 3 M aq. potassium hydroxide and the potassium perchlorate was removed by centrifugation. The resulting supernatant was freeze-dried and the residue redissolved in 10 mM hydrochloric acid (750 µl) for chromatography. It was sometimes not necessary to concentrate the supernatant by freeze-drying; in this case an aliquot of the final supernatant was chromatographed directly.

For *in vitro* metabolism experiments, the metabolism was stopped by addition of 0.6 M perchloric acid containing unlabelled carrier substances. The workup procedure was then carried out as described above.

Chromatographic separation and quantification of metabolites was done on silica gel t.l.c. plates (0.25 mm, 20 × 20 cm). An aliquot (up to 500 µl) of the sample to be analysed was applied along the baseline of the plate. In addition, a mixture of authentic standards was spotted at each side of the plate which was then developed in chloroform-methanol-0.880 ammonia (10:5:1 by volume, modified from Aures, Fleming, & Håkanson, 1968). The standards were visualized with aqueous potassium iodine plateate and the plate divided from solvent front to below baseline in 6 mm wide bands parallel with the baseline. The silica gel in each band was scraped off into a counting vial, digested in water (400 µl) and 40% aq. hydrofluoric acid (400 µl) and counted in a suitable scintillation fluid such as NE 260 (Nuclear Enterprises, Edinburgh). The radioactivity in each vial was presumed to be due to the authentic standard substance with which it was co-chromatographic.

R_F's of the standards in this system are histidine 0.09, imidazoleacetic acid 0.17, methylimidazoleacetic acid 0.26, histamine 0.47 and methylhistamine 0.72.

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