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Conversion of [4,5-^{3}H]leucine into ^3H_2O and tritiated metabolites in rat brain tissue. Comparison of a peripheral and intracranial route of administration

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It has been suggested, that the availability of amino acids is one of the major control mechanisms of cerebral metabolism[8]. Because of its frequent occurrence in most proteins, leucine is widely used as a precursor in studies on protein synthesis both *in vivo* and *in vitro*. Being an essential amino acid in mammals its supply to the cells should at least in part take place by uptake via the circulation. Indeed, uptake of L-leucine into brain tissue *in vivo*, either following central or peripheral administration appears to be rapid and efficient[2,9,12,13]. The same holds for the uptake of L-leucine into brain slices[7,8].

The availability of [^{3}H]leucine with high specific activity enables the labelling of brain proteins *in vivo* without notable disturbances of the endogenous pool. However, several studies indicated that [^{3}H]leucine undergoes a rapid and considerable conversion into metabolites and ^3H_2O in brain tissue following peripheral administration[1,12]. In the present study the conversion of L-[4,5-^{3}H]leucine into [^{3}H]non-amino acids and ^3H_2O, and the non-metabolized [^{3}H]leucine, were measured. The study was performed in brain stem tissue both after intradiencephalic and subcutaneous injection, using various periods of labelling.

Male, albino Wistar rats of an inbred strain, weighing 110–120 g were used. The rats were injected either with 60 μCi [4,5-^{3}H]leucine (47 Ci/mmole, The Radiochemical Centre, Amersham, U.K.) dissolved in 10 μl saline directly into the diencephalon by the method of Valzelli[14], or with 100 μCi of the [4,5-^{3}H]leucine in 0.2 ml saline, subcutaneously.

At 5, 10, 20 min or at 1, 2, 6 or 24 h after injection, the rats were killed by decapitation and the brain stems were dissected[5] within 2 min, rapidly washed twice in 5 ml of ice-cold 0.01 *M* sodium phosphate buffer pH 7.6 and homogenized with 3 ml using a tightly fitted Potter–Elvehjem homogenizer. Treatment of the homogenate with perchloric acid (final concentration 0.25 *M*) resulted in an acid-insoluble fraction containing labelled proteins[13] and an acid-soluble fraction containing the free amino acids, its metabolites and water. The ^{3}H-labelled material of the acid-soluble precursor pool was separated into 3 fractions by chromatography[10]. Briefly, an aliquot of the

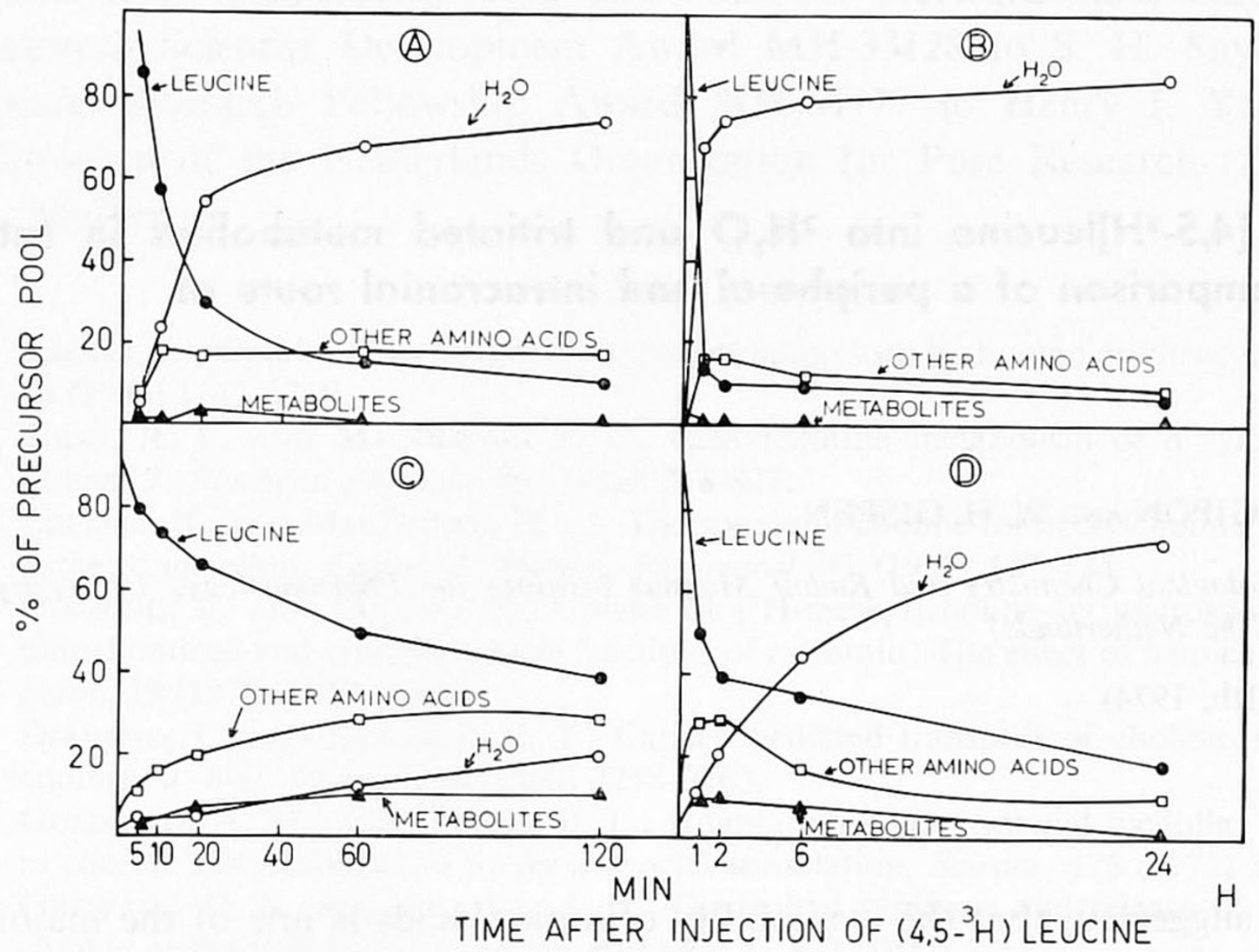

Fig. 1. Rats were injected with [^{3}H]leucine either subcutaneously (A and B) or directly into the diencephalon (C and D) and killed after various times of incorporation. Brain stems were dissected, homogenized and the acid-soluble cell fraction was separated into the fractions indicated in the figures, by passage through Dowex columns and by paper chromatography. The total radioactivity in each fraction is presented as a percentage of the radioactivity in the whole acid-soluble pool. A and B: subcutaneous injection; C and D: intradiencephalic injection. A and C show the short term events ranging from 5 to 120 min. B and D show longer term kinetics ranging from 1 to 24 h.

0.25 *M* perchloric acid supernatant was applied to a 25 × 6 mm column of Dowex 50 W X12 (200–400 mesh, H^+-form). Leucine and other amino acids were bound quantitatively onto the column[3,6,10,13], while tritiated water and the non-amino acids were eluted with water. The latter eluate was further fractionated on a column of Dowex 1 W X8 (200–400 mesh, OH^-) into tissue water and non-amino acids, with water and 2 *M* HCl respectively. The fraction containing the amino acids was eluted from the Dowex 50 W X12 column with 5 *M* NH_4OH and was further fractionated by descending paper chromatography, with *n*-butanol: benzyl alcohol (1:1) saturated with 0.067 *M* borate buffer pH 8.4 containing 0.067 *M* potassium chloride at 26 ± 1° for 36 h on Whatman 3 MM paper. By this method, leucine, isoleucine and phenylalanine were separated from each other and from the rest of the amino acids.

Radioactivities in the fractions eluted from the Dowex columns and eluted from the Whatman 3 MM paper, were measured in a Mark II Nuclear Chicago liquid scintillation counter using a Triton–toluene scintillation mixture[5]. [U-^{14}C]Leucine was added as an internal marker to check the recoveries of the Dowex fractionation, the paper chromatographic separation and to indicate the exact location of leucine.

The behaviour of the radioactive materials other than [^{3}H]leucine in chromatographic and electrophoretic systems, indicated that the radioactivity was mainly in the dicarboxylics[1]. This material was referred to as 'other amino acids', although a small contamination with labelled amines could not be excluded.

In Fig. 1 the relative amounts of radioactive constituents of the acid-soluble precursor pool are presented both after subcutaneous injection (Fig. 1A and B) and after intradiencephalic administration (Fig. 1C and D) of the precursor. To visualize the time sequence of the conversion of the administered [^{3}H]leucine into ^3H_2O and ^{3}H-metabolites adequately, two time axes were used. One ranging from 5 to 120 min (Fig. 1A and C) showing the short term events, the other one ranging from 1 to 24 h (Fig. 1B and D) for longer term kinetics. The values in Fig. 1 represent the means of determination on two rats. There was hardly any difference between duplicate determinations.

The amount of radioactivity present in leucine at the end of the incorporation period depended somewhat on the commercial batch used; it varied between 85 and 93 % for a 5 min incorporation period[13].

The appearance of ^{3}H-radioactivity in the 'other amino acids' and in the non-amino acids in brain stem is the same or slightly lower in case of the peripheral injection (Fig. 1A and B) as compared to the intracranial application (Fig. 1C and D). In contrast, after intracranial injection the amount of ^3H_2O was much lower. The dramatic transfer of label to ^3H_2O following subcutaneous injection corresponds with observations made by Banker and Cotman in mice, after intravenous injections[1].

For incorporation periods up to 5 min the conversion of the precursor does not interfere with the determination of the incorporation rate for both routes of administration. However, for short incorporation periods, the specific activity of brain stem proteins is extremely low after subcutaneous administration in comparison to that after intracranial injection (*e.g.* 1 % after a period of 5 min, Schotman, unpublished data).

Bearing in mind the presence of radioactive metabolites, this communication showed that for short incorporation periods the interference by ^3H_2O with rates of incorporation may be overcome by using an intracranial route of administration of the [^{3}H]leucine.

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