Spontaneous and evoked release of newly synthesized [^{14}C]-GABA from rat brain slices

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After local perfusion of the monkey brain *in vivo* with [^{14}C]-glucose, newly formed [^{14}C]-amino acids, including GABA can be detected (De Feudis, Delgado & Roth, 1969). No method, however, for monitoring continuous release of newly synthesized amino acids *in vitro* has been reported. The present technique was devised to measure the release of newly synthesized [^{14}C]-GABA from brain slices during perfusion with radioactive pyruvate as precursor.

Rat cortical slices, chopped in two directions at 0.3 mm intervals, weighing 100 mg, were placed in a Perspex tissue chamber and perfused at 37°C with Krebs bicarbonate medium containing [2-^{14}C]-pyruvic acid (specific activity 10 mCi/mmol), 2 μCi/ml, at a rate of 6 ml per hour. Perfusate samples were collected at 10 min intervals and stored on ice. At the end of the perfusion (90-150 min) the slices were homogenized in 0.1 N HCl and after centrifugation the supernatant fraction was used to estimate [^{14}C]-GABA remaining in the slices. In both perfusate and tissue samples [^{14}C]-GABA was estimated by the following procedure. Unchanged [2-^{14}C]-pyruvic acid and acidic products, including acidic amino acids, were removed by retention on a Dowex AG 1-X2 (CH_3COO^- form) anion exchange resin and the amino acid containing effluent collected. Basic and neutral compounds in the effluent, including [^{14}C]-GABA, were retained on an Amberlite CG 120 (H^+ form) cation exchange resin, washed with water and eluted with 1 M triethylamine in 50% methanol. [^{14}C]-GABA in dried eluate samples was selectively isolated by enzymic conversion to [^{14}C]-succinic acid using a bacterial enzyme preparation as described by Hall, Bownds & Kravitz (1970). The reaction mixture was then passed again over an Amberlite CG 120 (H^+ form) cation exchange resin. Basic and neutral amino acids were unaffected by the enzyme reaction and were retained by the resin, [^{14}C]-succinic acid formed from [^{14}C]-GABA was collected in the effluent and radioactivity determined by liquid scintillation spectrometry.

The spontaneous release of newly synthesized [^{14}C]-GABA increased during the first 40 min of perfusion, after which a steady state was maintained. Contaminants derived from [2-^{14}C]-pyruvic acid in blank samples accounted for less than 10% of radioactivity in the [^{14}C]-succinic acid samples. Raising the potassium concentration in the perfusing medium to 47 mM produced a 229% ± 30% (mean ± s.e. mean, $n = 4$) increase in release. The ratio of radioactivity remaining in the tissue at the end of perfusion, to the average basal efflux per 10 min collection period was 6.5 ± 0.9 (mean ± s.e. mean, $n = 7$). In contrast, when cortical slices were incubated with [^{3}H]-GABA for 30 min, followed by a washout perfusion, the amount of radioactivity remaining in the tissue after 60 min was over 100 times the average basal efflux per 10 min collection period.

Newly synthesized [^{14}C]-GABA may thus be released from a much smaller tissue pool than that labelled by [^{3}H]-GABA taken up from the external medium.

C.G. was supported by grants from the Royal Society and the CIBA Foundation. T.M.J. is an M.R.C. Scholar.

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