Urea Synthesis in the Living Rat Brain

In previous communications from this laboratory, the synthesis of urea-^{14}C from arginine-^{14}C was reported to occur in the living rat brain[1,2]. In these experiments only the rat brains were analysed for their radioactive urea content, and the objection was raised that perhaps these levels did not indicate endogenous brain urea synthesis, but were merely a reflexion of blood ^{14}C-urea levels, especially since it is known that urea readily diffuses across the blood brain barrier. Experiments were therefore designed to show that the brain ^{14}C-urea levels were independent of blood ^{14}C-urea levels and that the brain had the capacity to synthesize urea from arginine. This communication describes such experiments.

Guanido-^{14}C labelled L-arginine-hydrochloric acid was obtained through the courtesy of the California Corporation for Biochemical Research before it was commercially available and was further purified by descending paper chromatography in two consecutive solvent systems. Solvent 1: pyridine/acetic acid/water (50 : 35 : 15). Solvent 2: ethanol/water/diethylamine (76 : 23 : 1). Identification was determined using the ninhydrin spray[3], and a modification of the Sakaguchi test[4] in conjunction with coincidence of radioautography. The R_F of the arginine in the above two solvent systems was 0·40 and 0·46 respectively. The purity of the arginine-^{14}C was verified by chromatography of the arginine eluate from the second solvent system in separate solvent systems of 2,6-lutidine/collidine/water/diethylamine (1 : 1 : 1 : 2 per cent v/v), propanol/acetic acid/water (75 : 5 : 20) and 2,4-lutidine/collidine/water/diethylamine (1 : 1 : 1 : 2 per cent v/v), propanol/acetic acid/water (75 : 5 : 20) and 2,4-lutidine/collidine/acetic acid/water (1 : 1 : 5 per cent v/v : 2).

A 90-day-old male albino rat (Sprague–Dawley strain) was lightly anæsthetized with diethyl ether and the exposed saphenous vein was cannulated with a heparinized, polyethylene cannula. 5 min. later an intracisternal injection of 0·4 mgm. of guanido-^{14}C labelled L-arginine-hydrochloric acid was administered using a micro-calibrated syringe (total activity 10 μc. = 7·3 × 10^6 counts/min.). At intervals following the injection, 0·5 ml. samples of blood were withdrawn from the vein and analysed for the specific activity of radioactive urea. 60 min. after the injection, the animal was killed by decapitation and the brain analysed for radioactive urea. As a control for possible injection of urea-^{14}C as a contaminant of the arginine preparation as well as a control for urea formation from arginine as a result of the procedures employed, a litter-mate rat was killed and to the isolated brain was added 0·4 mgm. of guanido-^{14}C labelled L-arginine-hydrochloric acid. The brain was treated as described here.

Following the withdrawal of the blood samples from the experimental animal, 5 ml. of 15 per cent trichloracetic acid (TCA) were added and the samples were homogenized. 8 mgm. of unlabelled carrier urea were then added to the homogenates; the samples were centrifuged and the supernatant solutions were decanted and saved. The TCA from these fractions was then extracted with carbon tetrachloride. The samples were then taken almost to dryness with a rotary evaporator and the insoluble dixanthydrol derivative of urea was prepared and recrystallized to constant specific activity. The brain was homogenized and urea was isolated from the acid-soluble fraction by ion-exchange column chromatography. A derivative was prepared as described here.

Table 1. Specific Activity of Blood* and Brain* Urea Samples

Sample	Time (min. after injection)	Specific activity of urea (counts/min./mgm. urea)
Blood 1	18	3 × 10^3
2	20	3 × 10^3
3	30	3 × 10^3
4	40	5 × 10^3
5	50	6 × 10^3
6	57	7 × 10^3
Brain	60	250 × 10^3

* The whole rat brain contains 0·5 mgm. urea, and 0·5 ml. rat blood contains 0·29 mgm. urea.

Analyses for radioactivity and urea content were performed in a manner reported previously[1].

The results presented in Table 1 show that the specific activity of the brain urea is at least thirty times as great as the highest blood urea specific activity. The maximum urea-^{14}C contamination as determined by the specific activity of urea in the control brain is 6 × 10^3 counts/min./mgm. urea. If the brain urea were derived only from exogenous sources and was transported to the brain via the blood, then the brain urea specific activity could not be higher than that of the blood[5]. The fact that it is many times greater than the blood level, at all intervals in which blood was collected, is proof of endogenous brain urea synthesis from arginine.

Note: Ratner *et al.* have shown recently in partially purified rat-brain proteins that arginase is present; this is verified by our *in vivo* work[6].

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Antibody Response in Rabbits following Injection of Sheep Erythrocytes into Lateral Ventricle of Brain

Immunological studies of the cerebrospinal fluid have been confined mainly to the passage of antibodies from plasma into the cerebrospinal fluid[1]. However, the investigation of exchange between cerebrospinal fluid and blood has revealed that various electrolytes[2], dyes[3] and proteins[4] may find their way from cerebrospinal fluid into general circulation. Furthermore, some data[5] indicate that red blood cells cross cerebrospinal fluid-blood barrier. It seemed, therefore, advisable to approach the immunological sequences following the injection of heterologous erythrocytes into lateral ventricle of the brain.