Short Communications

Glutamate Uptake by Chick Retina

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The uptake of glutamate was found to be via a single high-affinity transport mechanism with K_m values of 35 and 95 μM for chick-embryo and mature chick retina respectively. These data contrast with the uptake of γ -aminobutyrate which in the same tissue has previously been shown to display two kinetically distinct mechanisms in the embryo, but a single low-affinity process in the mature retina.

Both glutamate and γ -aminobutyrate are strong candidates as transmitters in the central nervous system (Krnjevic, 1970). It is widely believed that the physiological action of these amino acids at the synapse is terminated by their rapid removal into surrounding cells (Iversen & Neal, 1968; Balcar & Johnston, 1973). The uptake properties of γ -aminobutyrate and glutamate have been studied in mature rat retina (Starr & Voaden, 1972; Goodchild & Neal, 1973; Neal *et al.*, 1973). The uptake mechanism for each of the amino acids was shown to possess a relatively high affinity for its substrate, with a K_m of the order of 10 μM .

We have demonstrated that the γ -aminobutyrate-uptake system of chick retina displays considerable differences from that of the rat retina. For instance, in the retina of chick embryo two kinetically distinct uptake processes were observed, one of which had a high affinity and the other a low affinity for its substrate (Tunncliff & Ngo, 1975; Tunncliff, *et al.*, 1975). In the post-hatched bird, however, only the low-affinity system was detectable. The disappearance with maturity of the high-affinity uptake mechanism for γ -aminobutyrate has been reported by Levi (1970) to occur also in slices of chick brain.

The present study was undertaken to determine how the uptake process for glutamate in chick retina compares with that of the γ -aminobutyrate-uptake mechanism in the same tissue.

Experimental

Animals. White Leghorn chicks of either sex were used for this investigation. Fertilized eggs were obtained from the Department of Poultry Science,

University of Saskatchewan, at the appropriate age. When retinas from mature birds were required, 1-day-old chicks were obtained and reared on starter ration until maturity.

Materials. L-[U- ^{14}C]Glutamic acid (specific radioactivity 260 mCi/mmol) was obtained from Amersham-Searle, Arlington Heights, Ill., U.S.A.

Krebs-Ringer phosphate buffer contained the following: 118.5 mM-NaCl; 4.75 mM-KCl; 1.8 mM- CaCl_2 ; 1.2 mM- MgSO_4 ; 16 mM-sodium phosphate buffer (pH 7.4); 1 g of D-glucose/litre.

Uptake of [^{14}C]glutamate. The bird was decapitated and the retina rapidly removed from each eye. The tissue was cut into pieces weighing approx. 3 mg each and preincubated in 5 ml of Krebs-Ringer buffer at 25°C for 10 min in a shaker water bath. [^{14}C]Glutamate (10 μl) was added to the medium, making the final concentration of the amino acid 50 μM . After a 10 min incubation the tissue was removed with forceps and washed three times in Krebs-Ringer buffer. The retina was lightly dried on filter paper and weighed. Finally it was transferred to a scintillation vial containing 0.5 ml of 80% (v/v) methanol, and after 30 min 10 ml of Bray's (1960) counting fluid was added and the radioactivity measured in a Nuclear-Chicago Isocap/300 liquid-scintillation counter. For the experiments on the effects of glutamate concentration on uptake the data were expressed in terms of dry weight of tissue.

Results

Uptake of [^{14}C]glutamate against time. To establish the time-period over which the uptake of glutamate was linear, retina from both embryonic (15-day) and mature birds was incubated in 50 μM -[^{14}C]glutamate at 25°C for up to 90 min. The results clearly indicated

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Fig. 1. Glutamate uptake as a function of Na^+ concentration in the medium (a) and effects of temperature on glutamate uptake (b)

(a) Embryo retina (○) and mature retina (●) were incubated in $50 \mu\text{M}$ - ^{14}C glutamate under the usual conditions except the Na^+ content was varied by the addition of different amounts of NaCl. Each point is the mean of three determinations. (b) Embryo retina (○) and mature retina (●) were incubated as described under 'Experimental', but the temperatures used were 10°, 15° or 25°C. Each point corresponds to the mean of three determinations.

that the uptake by embryonic retina was linear for up to 30 min and that by mature retina for up to 15 min. The final tissue/medium ratio (nCi of ^{14}C glutamate/g of tissue : nCi of ^{14}C glutamate/ml of medium) for the embryo was almost 50:1 and for the tissue from adult animals 60:1. In all subsequent experiments incubations were carried out for 10 min.

A preliminary experiment was carried out on mature retina under the usual conditions. Tissue was incubated for 10 min. The tissue was then homogenized in 100 μl of 0.1 M-HCl, and 50 μl of 10% (w/v) trichloroacetic acid was added. The solution was centrifuged for 10 min at 3000 rev./min. The supernatant was applied to a thin-layer plate with a cellulose support, and the chromatogram developed in butanol-acetic acid-water (8:5:3, by vol.). A single radioactive spot was detected that had the same R_f value as authentic glutamate.

Effects of Na^+ . Incubations of embryonic and mature retina were carried out in Krebs-Ringer buffer that contained $50 \mu\text{M}$ - ^{14}C glutamate and in which the phosphate buffer was replaced by 50 mM-Tris-HCl, pH 7.4. The Na^+ content of the medium was regulated by the addition of various amounts (0–120 mM) of NaCl. The Cl^- content was kept constant by adding appropriate amounts of choline chloride. The rate of glutamate uptake increased in parallel with an increased Na^+ content of the medium (Fig. 1a).

Influence of temperature on uptake. Retina from 15-day embryos and from mature chicks was incubated in the presence of $50 \mu\text{M}$ - ^{14}C glutamate for 10 min at 5°, 10° or 25°C. Fig. 1(b) shows that the uptake process for glutamate in both embryonic and mature retina is temperature-dependent.

Effect of substrate concentration on ^{14}C glutamate uptake. Under the standard conditions, retina from embryonic and mature animals was incubated in ^{14}C glutamate. A concentration range of 1 mM–10 μM was established by the addition of non-radioactive glutamate. The data were recorded by the method of Lineweaver & Burk (1934), shown in Fig. 2. The uptake of glutamate for both retinas showed a single straight line. The apparent K_m was 34.6 and 95.0 μM for embryo and mature retina respectively. The $V_{\max}$ was calculated to be 3.50 $\mu\text{mol}/\text{min}$ per g dry wt. for embryonic and 6.95 $\mu\text{mol}/\text{min}$ per g dry wt. for mature retina.

Discussion

The current data reveal that the glutamate-uptake systems of embryonic and mature chick retina are fundamentally the same. Both processes exhibit a relatively high affinity for glutamate of the order of 10–100 μM . The K_m of the embryo system is three times less than that of the mature tissue, and the value for the $V_{\max}$ is half that of the mature tissue.

Fig. 2. Lineweaver-Burk plot of the rate of uptake against glutamate concentration

(a) Embryo retina (15-day) was incubated in Krebs-Ringer buffer containing [^{14}C]glutamate at 25°C for 10 min. Further details are described in the text. Each point represents the mean of three determinations. (b) Mature retina was incubated under the same conditions as in (a). Each point is the mean of three determinations.

However, these differences are comparatively slight and might be expected of any developing biochemical system. For example, Johnston & Davies (1974) found a similar increase in both K_m and V_{max} for γ -aminobutyrate uptake by slices of rat cerebral cortex. Levi (1972) reported that in developing chick brain the V_{max} for glutamate uptake increased with maturity whereas the K_m remained constant. An increase in the number of uptake sites at the synapse with development is a plausible explanation for the increase in V_{max} . Indeed, it has already been demonstrated that, in cultures of chick spinal cord undergoing synaptogenesis, the rate of glutamate uptake significantly increases in parallel (Cho *et al.*, 1973).

What emerges very clearly from the present results is the marked difference in chick retina between the uptake mechanisms for glutamate and γ -aminobutyrate. The glutamate-uptake system displays a high affinity for its substrate in both embryonic and mature retina, whereas the uptake system for γ -aminobutyrate differs between the embryo and the adult (Tunnicliff *et al.*, 1975). The embryo exhibits two kinetically distinct phases, one with a K_m of about $100\ \mu\text{M}$ (low-affinity) and the other of about $10\ \mu\text{M}$ (high-affinity), whereas the retina from post-hatched birds possesses a low-affinity uptake system only. Unfortunately no explanation can be offered for this phenomenon, but Levi (1970) has found that the development of the γ -aminobutyrate-uptake system in chick brain is extremely similar to that of chick retina.

Several workers have claimed that in the mature rat nervous system, including the retina, the uptake of glutamate is via a biphasic mechanism with two distinct values for the K_m (Logan & Snyder, 1971;

Balcar & Johnston, 1973; Neal *et al.*, 1973). The processes they describe are similar to that for γ -aminobutyrate in chick-embryo retina (Tunnicliff *et al.*, 1975). It is apparent from the present data that the uptake system for glutamate by chick retina exhibits different properties from that in the nervous system of other species. The significance of these differences to the organism is hard to assess, however, for it is not known what physiological importance can be attached to a combined high- and low-affinity transmitter-uptake system.

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