The Influence of the Intracellular Concentration of Sodium on the Uptake of L-[¹⁴C]Valine by Chopped Tissue from Cerebral Cortex

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1. The intracellular concentrations of sodium, potassium, ATP and creatine phosphate in chopped tissue from guinea-pig cerebral cortex were altered by a 10 min period of electrical stimulation. 2. The ability of the tissue to take up L-[¹⁴C]valine and incorporate it into protein was tested at various times after the cessation of electrical stimulation. 3. The restoration of the ability of the tissue to accumulate L-[¹⁴C]valine correlated closely with the restoration of the resting intracellular concentrations of sodium and ATP.

Previous work has shown that there is a Na⁺-dependent uptake of [¹⁴C]valine into chopped tissue from cerebral cortex (Jones & Banks, 1970*b*). In that study there was a suggestion that the uptake of valine was more closely coupled to the transmembrane concentration gradient of Na⁺ than to the intracellular concentration of ATP and creatine phosphate.

The involvement of Na⁺ in uptake processes is generally inferred from the effects of altering the Na⁺ concentration of the external medium or by inhibiting the Na⁺ pump with ouabain (Stein, 1968; Crane, 1965; Christensen, 1967). However, under physiological conditions the uptake is more likely to be altered by changes in the intracellular concentration of Na⁺ than by variations in the Na⁺ content of the extracellular fluid. The object of the present experiments was to increase the intracellular concentration of Na⁺ by short periods of electrical stimulation and subsequently to correlate the ability of the tissue to accumulate radioactive valine with the restoration of its ion and high-energy phosphate contents. The experiments also provided an opportunity to examine the correlation between the intracellular concentration of labelled valine and its incorporation into protein.

METHODS

The tissue used in this study consisted of small blocks (0.35 mm × 0.5 mm × 0.5 mm) of superficial cerebral cortex of male guinea pigs (375–425 g) prepared as previously described (Jones & Banks, 1970*a*). The small blocks of

tissue were incubated in Krebs phosphate saline (see Jones & Banks, 1970*a*), pH 7.4, containing 0.1% inulin in oxygenated Warburg flasks at 37°C for 20 min, after which the respiratory rate of the tissue was measured for 30 min. After this, electrical pulses (18 V; 100 pulses/s; time-constant, 0.4 ms) were applied to the chopped tissue (see Jones & Banks, 1970*a*) for 10 min. Immediately on stopping or a definite time after stopping, electrical stimulation, 1 μCi of L-[U-¹⁴C]valine (260 mCi/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.) was added to the tissue suspension to give a final valine concentration of 1.1 μM. At the end of the incubation the tissue blocks were filtered under vacuum on Whatman no. 40 filter paper placed on Micro-Hirsch funnels. The blocks of cerebral cortex were lifted from the filter paper with a stainless-steel spatula, homogenized in 3 ml of 5% (w/v) trichloroacetic acid and centrifuged at 1500g for 5 min. The supernatant from this, and from a further 3 ml washing with 5% trichloroacetic acid, was used to determine the uptake of [¹⁴C]valine (Jones & Banks, 1970*b*). Previous work had shown that [¹⁴C]valine was the only radioactively labelled compound present in the acid-soluble fraction at the end of the incubation (Jones & Banks, 1970*a*). The uptake of [¹⁴C]valine was expressed in terms of a concentration ratio defined as:

$$\frac{\mu\text{mol of intracellular } [^{14}\text{C}]\text{valine/g of tissue}}{\mu\text{mol of } [^{14}\text{C}]\text{valine/ml of saline}}$$

The protein pellet was treated as previously described (Jones & Banks, 1970*a*) and the incorporation of radioactivity into protein determined.

ATP, creatine phosphate and intracellular Na⁺ and K⁺ concentrations were determined (Jones & Banks, 1970*a*) in chopped tissue that had been incubated and stimulated in exactly the same manner as that used for the experiments on the uptake and incorporation of [¹⁴C]valine into protein.

The results are expressed as means ± s.e.m. with the numbers of determinations in parentheses.

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RESULTS

Effect of electrical stimulation on Na^+ , K^+ , ATP and creatine phosphate content of the tissue. Chopped tissue from cerebral cortex was stimulated for 10 min and the Na^+ , K^+ , ATP and creatine phosphate contents determined at various times during the subsequent 50 min (Figs. 1 and 2). At the onset of stimulation the values for intracellular Na^+ and K^+ were respectively 23.5 ± 5.5 (4) and 60.7 ± 4.6 (4) $\mu\text{equiv./g}$ wet wt. of tissue; at the end of the period of electrical stimulation the corresponding values were 57 ± 4.8 (4) and 38.9 ± 2.6 (4) $\mu\text{equiv./g}$ wet wt. of tissue. It was 20 min after the cessation of electrical stimulation before the Na^+ concentration returned to normal and rather longer before the intracellular K^+ recovered its initial value. These results are similar to those obtained by McIlwain and his colleagues (McIlwain, 1966). The concentrations of both ATP and creatine phosphate fell during the period of electrical stimulation. The ATP concentration recovered within 5–10 min of the

cessation of electrical stimulation; although the creatine phosphate content of the tissue rose rapidly it never completely returned to its control value.

Recovery of [^{14}C]valine uptake after 10 min electrical stimulation. In these experiments [^{14}C]valine was added to the incubation medium immediately after, or a definite time after, electrical stimulation had ceased. When labelled valine was added immediately after electrical stimulation (i.e. 0 min recovery) both the initial rate of uptake and the amount of label finally accumulated were very much lowered. Thus after 30 min incubation with [^{14}C]valine the concentration ratios in the control and electrically stimulated tissue were 5.7 ± 0.27 (3) and 1.88 ± 0.21 (3) respectively. After a 10 min period of recovery, the initial rate of uptake of label had returned to the control value (Fig. 3). How-

Fig. 1. Recovery of the intracellular ion concentration of chopped tissue from cerebral cortex after a 10 min period of electrical stimulation (indicated by horizontal bar). (a) Na^+ ; (b) K^+ . $\square$, Control tissue; $\blacksquare$, electrically stimulated tissue (18 V; 100 pulses/s; time-constant, 0.4 ms). Each point is the mean $\pm$ s.e.m. (represented by vertical bars) of four determinations.

Fig. 2. Recovery of the concentration of ATP and creatine phosphate in the tissue after a 10 min period of electrical stimulation (indicated by horizontal bar). (a) ATP; (b) creatine phosphate. $\square$, Control tissue; $\blacksquare$, electrically stimulated tissue (18 V; 100 pulses/s; time-constant, 0.4 ms). Each point is the mean $\pm$ s.e.m. (represented by vertical bars) of three determinations.

Fig. 3. Recovery of the uptake of [^{14}C]valine by chopped tissue from cerebral cortex after a 10 min period of electrical stimulation. Recovery times allowed after electrical stimulation, before the addition of [^{14}C]valine, were: (a) 0 min; (b) 2 min; (c) 5 min; (d) 10 min; (e) 20 min; (f) 40 min. $\square$, Control tissue; $\blacksquare$, electrically stimulated tissue (18 V; 100 pulses/s; time-constant, 0.4 ms). Each value is the mean $\pm$ s.e.m. (represented by vertical bars) of three determinations.

ever, the final concentration ratio obtained after 30 min incubation in the presence of the labelled valine returned to the control value only after a recovery period of 20 min (Fig. 3).

The gradual decline in the ability of the control slices to take up [^{14}C]valine during increasingly long incubation times is possibly related to a general deterioration in the tissue, as is suggested by a gradual fall in its creatine phosphate content (Fig. 2).

Recovery of the incorporation of [^{14}C]valine into protein after 10 min electrical stimulation of chopped tissue from cerebral cortex. After electrical stimulation of the slices the incorporation of [^{14}C]valine into protein was inhibited irrespective of whether the tissue was allowed no time or up to 40 min to recover from electrical stimulation (Fig. 4). When the electrically stimulated tissue was allowed no recovery time before the [^{14}C]valine was added, incorporation into protein was markedly

lowered; incorporation into the control and electrically stimulated slices being 2500 ± 88 (3) d.p.m./mg of protein and 1282 ± 144 (3) d.p.m./mg of protein respectively after a 30 min incubation (Fig. 4a). The ability of the tissue to incorporate labelled valine into protein gradually increased with increasing recovery time up to 10 min, but thereafter declined (Fig. 4). This decline in the rate of incorporation of label into protein was not related to any diminution in the ability of the tissue to take up [^{14}C]valine. Indeed, the ability to accumulate label was much greater after a 40 min period of recovery than after a 10 min period of recovery (see Fig. 3).

DISCUSSION

It is widely held that the uptake of amino acids into many types of cells is dependent upon the concentration gradient of sodium across their plasma membranes (Vidaver, 1964a,b; Eddy, 1968;

Fig. 4. Recovery of the incorporation of [^{14}C]valine into the protein of chopped tissue from cerebral cortex after a 10 min period of electrical stimulation. Recovery times allowed after electrical stimulation, before the addition of [^{14}C]valine, were: (a) 0 min; (b) 2 min; (c) 5 min; (d) 10 min; (e) 20 min; (f) 40 min. $\square$, Control tissues; $\blacksquare$, electrically stimulated tissue (18 V; 100 pulses/s; time-constant, 0.4 ms). Each value is the mean $\pm$ S.E.M. (represented by vertical bars) of three determinations.

Christensen, 1967; Crane, 1967; Stein, 1968). Previous work on the uptake of [^{14}C]valine by brain tissue supported this view since uptake appeared to show a greater dependence upon the transmembrane gradient of sodium than upon the intracellular concentration of ATP (Jones & Banks, 1970b; see also Vidaver, 1964b). The present study was undertaken in an attempt to assess the relative importance of the sodium gradient and ATP in the uptake process; it differed from previous studies in that the Na^+ concentration gradient was altered by changing the intracellular concentration of Na^+ rather than by changing the Na^+ content of the suspending medium.

The results show that the uptake of [^{14}C]valine was impaired, both with respect to its initial rate and to the concentration ratio obtained finally, immediately after a period of electrical stimulation that had decreased both the sodium concentration gradient across the cell membranes and the intra-

cellular concentration of ATP. The ability of the tissue to accumulate labelled valine improved in both respects as the transmembrane Na^+ concentration gradient and ATP contents returned towards their control values during the period following the cessation of stimulation.

In the present study the intracellular ATP concentration of the stimulated tissue recovered before the Na^+ concentration. Therefore it is perhaps possible to distinguish between the action of intracellular ATP concentration and intracellular Na^+ concentration in determining the ability of the tissue to take up [^{14}C]valine. The initial rate of uptake of [^{14}C]valine took approximately the same time to recover as the intracellular ATP concentration, even though at this time the intracellular Na^+ concentration was still elevated. However, the ability of the stimulated tissue to maintain the same steady-state concentration of radioactive valine as the control tissue did not recover until the intra-

cellular Na^+ concentration had returned to its pre-stimulation value. Thus complete recovery of the pattern of uptake was not obtained until the control concentrations of intracellular Na^+ and ATP were regained.

The present set of results are therefore consistent with the view that the uptake of [^{14}C]valine is dependent upon the transmembrane gradient of Na^+ . They also suggest that, although the intracellular concentration of ATP may be less important than the intracellular Na^+ concentration in determining the maximal ability of the tissue to take up radioactive valine, it may play an important role in determining the initial rate at which radioactive valine can be taken up by the tissue.

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