stimulus, a small birefringence signal in response to the 2nd stimulus can be detected. Once the difference has been spotted the convex falling phase and the increased undershoot of the double stimulus response (bottom) compared to the single stimulus trace (top) are quite prominent. A tentative analysis of the full set of original traces in their figure 3a seems to indicate fair agreement of these values with our own data (see our figure 3).

Suarez-Kurtz and Parker[3] suggest that the early birefringence signal is caused by a process which is fully saturated during a twitch, like Ca-binding to troponin or latency relaxation. Under favourable conditions, however, the birefringence transients do summate (see figure 2e), they clearly precede latency releaxation[2,8], and birefringence signal and latency relaxation can be dissociated[9].

Therefore, in contrast to the interpretation presented by Suarez-Kurtz and Parker[3], we conclude that the results of double stimulation experiments do not disprove the hypothesis that the early large birefringence signal reflects a potential change of the sr membrane in response to the release of Ca from the sr. This hypothesis has been proposed[1] because potential changes across excitable membranes (muscle surface membrane[1,2], pike olfactory nerve[10], squid giant axon[11]) are known to produce corresponding birefringence signals and because muscle fibres stained with a fluorescent dye known to monitor membrane potential in nerve (indodicarbocyanine) produce a fluorescent transient with the same time course as the early large birefringence signal[12].

The observation that the signal caused by a 2nd stimulus at short intervals is too small to produce an obvious increase in total signal size (figure 1) is in agreement with the assumption that the longitudinal sr is calcium sensitive and undergoes a Nernst-potential change in response to the rapid change in myoplasmic $[Ca^{++}]$. The additional potential change in response to an immediately following 2nd release of Ca^{++} is then expected to be much smaller even if the same amount of Ca^{++} would be released which is probably not the case. This alternative model predicts the observed signal size better than the original assumption that the Ca-current is charging the membrane capacitance[1], where a potential change proportional to the amount of Ca^{++} released would be expected.

If a calcium sensitive longitudinal sr membrane is assumed to cause the signal, injection of EGTA into a muscle fibre would abolish the Nernst-potential change by preventing the rise in free myoplasmic $[Ca^{++}]$, in agreement with the observations by Suarez-Kurtz and Parker[3]. As these authors point out, a signal directly caused by the Ca-current charging the sr membrane should have remained unaffected, provided that EGTA does not interfere with the Ca-release mechanism.

In conclusion, therefore, the results of these series of experiments with double stimulation indicate that a change in sr potential has remained a likely candidate for the unknown process during ec-coupling causing the early large birefringence signal in skeletal muscle fibres.

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Thiamin turnover rate in some areas of rat brain and liver: A preliminary note[1]

G. Rindi, C. Patrini, V. Comincioli and C. Reggiani

Institutes of Human Physiology and Mathematics, University of Pavia, Pavia (Italy), 26 July 1978

Summary. Thiamin turnover rates in some nervous structures and liver of rats were evaluated in a steady state condition, using thiamin-^{14}C as a tracer. The radioactivity contents were analyzed by means of a mamillary type compartmental model. Excluding the liver, turnover rate values of the nervous structures were ordered in the following sequence: sciatic nerve (0.58 μg/g · h) > cerebellum > hypothalamus > midbrain > corpus striatum > cerebral cortex (0.16 μg/g · h).

The problem of thiamin function in the nervous system (coenzymatic and/or noncoenzymatic) is yet unresolved[2]. A useful approach might be a comparative study of thiamin turnover in brain areas, particularly those selectively affected by thiamin-deficiency[3]. This prompted us to perform an investigation on thiamin turnover in the nervous system, since no data are available in the literature. Here we will briefly give the results obtained on the rat brain, using labelled thiamin as a tracer in a steady-state condition. The liver was also included for comparison purposes, because of its thiamin storage function in the body. A complete account will be published elsewhere.

Materials and methods. 30 μg of [Thiazole-2-^{14}C]-thiamin (Radiochemical Center, Amersham, England: specific activity 14 mCi/mmole) were i.p. injected into female Wistar rats (230–280 g of b.wt), starved at night. Since the amount of thiamin injected corresponds to the rat's daily requirement[4], during the successive 24 h the rats were fed a thiamin-deficient diet (Dr Piccioni, Brescia, Italy), and then again a complete diet. In this way, thiamin intake and total thiamin organ contents were kept constant (steady state condition). The rats were sacrificed by decapitation at time intervals from 5 min to 96 h from thiamin injection. Different nervous structures (table) and a sample of liver were rapidly dissected in the cold. For brain dissection, the method of Glowinski and Iversen[5] was followed. Thiamin was extracted from the tissues with cold 0.5 N HCl, and 40% trichloroacetic acid was used for deproteinization. Labelled free and phosphorylated thiamin of the extracts were separated following Sharma and Quastel[6]. Thiamin radioactivity was determined by a Geiger-Müller low background flow-counter (Nuclear Chicago, mod. 512) after drying suitable samples on planchets. The efficiency of the counting was 81% and the error was less than 2%. Total (sum of free and phosphorylated) labelled thiamin radioactivity was expressed as nCi/g of wet tissue. Preliminary experiments showed a mean recovery of the labelled thiamin added to tissue samples of 97% (10 experiments). The

Total thiamin contents, fractional rate constants (FRC), turnover rates and time constants for thiamin efflux (K) in different body regions of rat

Structures	Total thiamin content[a] (μg/g wt)	Turnover rates[b] (μg/g·h)	Fractional rate constants[b] (h^{-1}) Afflux	Efflux	K[c] (h)
Cerebellum	4.37±0.07	0.55	0.447	0.13	7.9
Sciatic nerve	1.41±0.02	0.58	0.120	0.41	2.4
Medulla	2.90±0.14	0.54	0.225	0.18	5.3
Pons	3.27±0.11	0.45	0.227	0.14	7.2
Spinal cord	2.07±0.10	0.39	0.127	0.19	5.3
Hypothalamus	2.86±0.12	0.36	0.267	0.13	8.0
Midbrain	3.10±0.11	0.29	0.122	0.09	10.5
Corpus striatum	3.32±0.15	0.27	0.170	0.08	12.3
Cerebral cortex	2.61±0.15	0.16	0.090	0.07	16.4
Liver	9.07±0.76	0.95	0.267 0.172[d]	0.10	9.5

[a] Average of 25 determinations±SE. [b] A complete statistical analysis of the confidence limits of the parameters shall be reported in the in extenso paper. The range of the calculated SD was found to be 5–10% of the parameter values. [c] K = 1/FRC efflux. [d] From peritoneum (this value was not normalized to 1 g of tissue).

chemical determination of the unlabelled thiamin content was made by a modification of the fluorometric method of Burch et al.[7], using Takadiastase (Parke Davis, Detroit, Mich., USA) as a dephosphorylating agent. The mean recovery of the unlabelled thiamin added to tissues was 98% (8 experiments).

The results of the radioactivity measurements were analyzed by means of a mamillary type compartmental model; 12 compartments, corresponding to the nervous structures, liver and peritoneum (which is considered the tracer entry compartment) were placed around a distributing central compartment. A direct connection was also assumed between liver and peritoneum. From this model a system of differential equations was derived and numerically resolved by a variable order-variable step formulation of Adams predictor-corrector method[8].

The numerical values of the model parameters were evaluated by minimizing a cost function, accounting for the marked differences of single regional curves, by a combination of the Levenberg-Marquardt with steepest-descent method[9]. These parameters, which can be considered as fractional rate constants, could be transformed into turnover rates by multiplying them by the total thiamin content of the single region[10].

Results and discussion. Cerebellum (table) is the brain area containing the highest amount of total thiamin, a result which is in accordance with that of previous authors[11,12]. In all the structures, excluding liver, the peaks of labelled total thiamin radioactivity were reached in about 24 h, their different amplitudes ranging from 12.92 nCi/g for cerebellum to 1.44 for sciatic nerve. Using the mathematical model, fractional rate constants were calculated both for thiamin afflux into single structures and for its efflux. The afflux constant represents the probability that a thiamin particle, coming from all body tissues, has to enter a given area. The cerebellum had the highest thiamin afflux constant, its value being superior than that of the liver. This means that the cerebellum, on a weight basis, has a greater 'avidity' for thiamin as the liver.

Efflux fractional rate constants of 0.41–0.06 h^{-1} correspond to time constants of 2.4–16.4 h. By multiplying the efflux fractional rate constant by thiamin regional concentration, the thiamin turnover rate could be calculated (table). Among brain areas, the cerebellum had also the highest thiamin turnover rate, followed by medulla and pons, having rates 82 and 98% respectively of that of cerebellum. On the contrary, the cerebral cortex had the lowest thiamin turnover rate (29% of cerebellum). Apparently the turnover rates are not strictly related to the thiamin content of a particular brain area (table), to the regional blood flow[13], or to glucose and O_2-utilization[14,15]. They might possibly be related to the cell (or neuron) number present in the individual area, the cerebellum having the highest cellular density[16]. However, the values of turnover rates are in good accordance with brain area sensitivity to thiamin deficiency. The pons and the cerebellum, which have the highest relative thiamin turnover rates, are the brain areas where thiamin deficiency causes a decrease of total thiamin (or thiamin pyrophosphate) content greater than in cerebral cortex[11,12,17], the area with the lowest thiamin turnover rate. It is also noteworthy that the earliest histological lesions by thiamin-deficiency were found in vestibular nuclei and other brainstem structures[18–20,21] as well as in cerebellum[3,21,22], where we found the highest thiamin turnover rates.

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