CALCIUM-BINDING PROPERTIES OF TISSUE PROTEINS AFTER KIDNEY ALLOGRAFTING

I. P. Ermakova, V. P. Buzulina, and I. A. Pronchenko

UDC 616.61-089.843-089.168-07:[616-008.924.1-008.61-02:616-008.939.6

KEY WORDS: tissues; tissue proteins; calcium-binding properties of proteins; kidney allografting.

Data on the existence of physicochemical nonhormonal tissue mechanisms of counteraction of hypercalcemia on the occasion of an excessive movement of calcium into the blood [4] and data on the content of proteins with calcium-binding properties in connective tissue and the matrix of bone tissue [5, 10] suggest that one cause of hypercalcemia and also of calcification of soft tissues and blood vessels, observed in recipients of an allografted kidney (AGK) [1] may be disturbances of calcium binding by tissue proteins.

The aim of the investigation described below, which was conducted on healthy individuals and patients with AGK, was to study the mechanism of calcium-binding by the tissues in response to an excessive intake of calcium, to determine the quantitative parameters of calcium-binding by the tissue substrate, and to discover the relationship between calcium-binding by the tissues and the level of secretion of calcium-regulating hormones.

EXPERIMENTAL METHOD

The process of calcium-binding by nonplasma (tissue) systems was investigated in 19 recipients of an AGK with satisfactory function of the transplant (plasma creatinine concentration 107 ± 21 μmoles/liter) and in 14 healthy volunteers, against the background of intravenous drip injection of calcium gluconate in 5% glucose solution for 156.8 ± 14.5 min. The quantity of calcium injected into the recipients of AGK and healthy individuals was similar (0.22 ± 0.04 and 0.26 ± 0.059 mmoles/kg body weight). On the day of the experiment, before breakfast and after deprivation of food for 12 h, the subjects drank 0.5 liter of bidistilled water. Before the beginning and during the calcium infusion every 30 min urine was collected, blood was taken from the cubital vein without application of a tourniquet, through a siliconized cannula into a syringe, rinsed with heparin (2 U/ml), and blood was taken from the capillaries of a finger, with an interval of not more than 5-10 min between collection of the urine and taking the blood. The total calcium (CaT) concentration in the samples of blood plasma and urine and the infused solution of calcium gluconate was determined on an atomic-absorption spectrophotometer (IL-151, USA), ionized calcium (Ca^{2+}) was determined in samples of blood plasma on an SS-20 analyzer ("Orion Research," USA), total protein was determined by the biuret method, albumin by electrophoresis on a cellulose acetate film; the pH and pCO_2 of plasma of venous and capillary blood on an AVL-940 gas analyzer (Switzerland). In samples of venous blood taken from some patients before the end of calcium infusion and immediately thereafter, levels of calcitonin (CT) and parathyroid hormone (PTH) levels were determined by an immunoreactive method, relative to the C-terminal fragment, using kits from "CIS" (France). In each investigation, the following parameters were calculated for each time interval: 1) CAT concentration in 1 liter of serum water in samples of plasma [7], and the Ca^{2+} concentration at pH and pCO_2 of the capillaries [2] in order to establish the concentration in blood flowing to the organs and tissues (Ca^{2+}_{cap}); 2) the quantity of calcium retained in the body during each period (ΔM_{ret}), as the difference between the quantity of calcium injected and the quantity excreted with the urine; 3) the total quantity of calcium retained in the body toward the

Research Institute of Transplantology and Artificial Organs, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. I. Shumalov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 9, pp. 256-258, September, 1991. Original article submitted October 20, 1990.

0007-4888/91/0009-1255$12.50

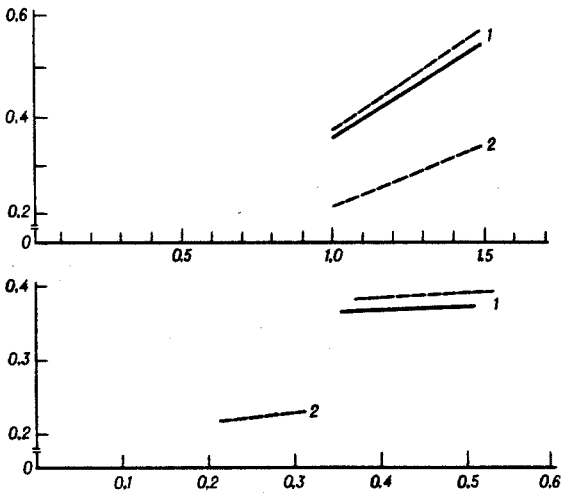

Fig. 1. Kinetics of calcium-binding by tissues in normal individuals and recipients of AGK with induced hypercalcemia in vivo. Above — between Langmuir coordinates: Ca^{2+} ion concentration in capillary blood plasma (Ca^{2+}_{cap}, in mmoles·liter^{-1}); ordinate, pool of exchangeable calcium in tissues (M_{tiss} in mmoles·kg^{-1}). Continuous line — in healthy individuals Yx = 0.383x — 0.026, Xo = +0.068 ± 0.066: r = 0.97 ± 0.03. Broken lines — in recipients of AGK: 1) Yx = 0.401x — 0.026; Xo = +0.063 + 0.044; r = 0.91 ± 0.057; 2) Yx = 0.25x — 0.035; Xo = +0.176 + 0.119; r = 0.91 ± 0.079. Below — Scatchard plot: abscissa, pool of exchangeable calcium in tissues (M_{tiss}, in mmoles·kg^{-1}); ordinate, ratio of pool of exchangeable calcium in tissues to Ca^{2+} concentration in capillary blood plasma (M_{tiss}/Ca^{2+}_{cap}, in liters·kg^{-1}). In healthy individuals Yx = 0.087x + 0.32; in recipients of AGK: 1) Yx = 0.135x + 0.31; 2) Yx = 0.17x + 0.183.

end of each period ($\Delta M_{tot\cdot rel}$), as the sum of ΔM_{rel} and ΔM_{tot} for the preceding period; 4) the total pool of exchangeable fasting calcium (Mm_{tot}) as the intercept (Yo) of the regression equation between the $\Delta M_{tot\cdot rel}$ series and the corresponding Ca^{2+}_{cap} concentrations (assuming that the coefficient of the regression equation is the same in hypo- and hypercalcemia); 5) the total pool of exchangeable calcium toward the end of each period ($\Delta M_{tot\cdot rel}$); 6) the calcium pool in the blood plasma toward the end of each period ($M_{pl\cdot rel}$), as the product of CaT concentration in the corresponding period and the circulating plasma volume, considered to be 4.5% of body weight [3], and stable throughout the period of investigation, since the hematocrit remained unchanged; 7) the calcium pool in the nonplasma (tissue) sector toward the end of each period ($M_{tiss\cdot rel}$/kg). The mechanism of calcium-binding by the tissues was assessed between Langmuir, Scatchard, and Hill coordinates [9]. The ability of the tissue buffer systems of the body to counteract hypercalcemia through a cooperative mechanism of binding was estimated relative to parameters obtained on the basis of individual regression equations between Langmuir coordinates (Fig. 1, top): 1) the pool of exchangeable calcium in the tissues when Ca^{2+}_{cap} = 1.0 mmole/liter (M_{tiss}/kg), and 2) the buffer capacity of the tissue buffers for calcium (β_{tiss}), reflected in the coefficient of regression. On the basis of these parameters, we also calculated kinetic binding constants at Ca^{2+}_{cap} = 1.0 mmole/liter: the effective association constant (K_a) and the effective number of calcium-binding interacting centers in the tissue substrate (h), according to the equations:

$$K_a = (M_{tiss}/kg)^2/\beta_{tiss} \text{ and } n = (M_{tiss}/kg)\cdot(1/K_a + 1).$$

The last terms were derived through transformation of equations describing the kinetics of the cooperative binding mechanism:

$$M_{tiss}/kg = K_a \cdot n \cdot [Ca^{2+}]^n/(1 + K_a[Ca^{2+}]^n) \text{ and } \beta_{tiss} = K_a \cdot n^2 [Ca^{2+}]^{n-1}/(1 + K_a [Ca^{2+}]^n)^2.$$

TABLE 1. Parameters of Calcium-Binding by Tissues at Ca^{2+} = 1 mmole/liter and Secretion of Calcium-Regulating Hormones in Recipients of AGK (M ± σ)

Parameter	Normal subjects	AGK (n = 19)	
		group 1	group 2
M_{tiss}, mmoles·kg^{-1}	0,357±0,087 (14)	0,375±0,045 (12)	0,218±0,056* (7)
β_{tiss}, liter·kg^{-1}	0,383±0,09 (14)	0,401±0,048 (12)	0,263±0,055* (7)
K_a	0,333±0,088 (14)	0,358±0,048 (12)	0,184±0,062* (7)
n	1,46±0,024 (10)	1,45±0,063 (12)	1,46±0,15 (7)
PTH_{bas}, pmoles·liter^{-1}	5,45±1,81 (111)	39,0±48,0* (11)	24,6±18,0* (5)
ΔPTH, pmoles·liter^{-1}	—	11,2±66,5 (8)	23,5±28,6 (2)
CT_{bas}, pmoles·liter^{-1}	0,69±0,39 (140)	4,02±2,68* (11)	2,93±0,98* (5)
ΔCT, pmoles·liter^{-1}	—	10,7±30,0** (8)	1,83±3,51 (5)

Legend. *p < 0.02 compared with normal subjects; **p < 0.05. Δ) Change in blood hormone concentration with basal level after end of calcium infusion; number of observations shown in parentheses.

EXPERIMENTAL RESULTS

Calcium-binding by the tissue substrate by means of a mechanism of positive cooperativeness was established in recipients of AGK and in healthy individuals: positive values of X0 in Langmuir coordinates, positive regression in Scatchard coordinates (Fig. 1), and Hill's coefficient over 1.0 (2.08 ± 1.06; r = 0.97 ± 0.02). In 12 recipients of AGK M_{tiss}/kg was normal (group 1), in seven it was depressed (group 2) (Table 1). β_{tiss}, effective K_a, and n in group 1 did not differ from those in healthy individuals; in group 2 a fall of M_{tiss}/kg was associated with a fall in β_{tiss} (by 35%) and K_a (by 43%). Basal secretion of PTH and CT in recipients of AGK was increased (Table 1), but no statistically significant differences were found either in the level of basal secretion of PTH and CT or in the level of change of their secretion toward the end of calcium infusion, between groups 1 and 2. Likewise, no correlation was found between M_{tiss}/kg and basal secretion of PTH, or between β_{tiss} and the degree of increase of CT secretion in the total group of AGK recipients (r = –0.08 and r = +0.33, respectively, p > 0.1).

Thus, in healthy individuals and in the absolute majority of AGK recipients a cooperative mechanism of calcium-binding by the tissues when injected by intravenous drip was established, evidence of the protein nature of the tissue substrate [9]. In 37% of AGK recipients, in whom the cooperative mechanism of binding was preserved, there was a marked decrease in the pool of exchangeable calcium in the tissues and of the buffer capacity of the tissue proteins, which was due to a reduction in the effective binding constant and did not depend on the level of secretion of calcium-regulating hormones. The disturbances of buffering of calcium by the tissues when its intake into the blood is increased may cause hypercalcemia after daytime consumption of food or calcium preparations and may be one cause of the pathological calcification of the vessels and soft tissues of patients after AGK, even if the transplant is functioning satisfactorily.

LITERATURE CITED

1. I. P. Ermakova, Sh. Sh. Shotemor, E. R. Levitskii, et al., Transportation and Artificial Organs [in Russian] (1984), p. 7.

2. A. L. Levin, M. Yu. Andrianova, I. P. Ermakova, et al., Byull. Éksp. Biol. Med., No. 12, 704 (1986).
3. M. H. Maxwell and C. R. Kleeman (eds.), Clinical Disorders of Fluid and Electrolyte Metabolism, New York (1980).
4. D. H. Copp, H. Moghadam, and E. D. Mensen, The Parathyroids, R. O. Greep and R. W. Talmage (eds.), New York (1961), p. 203.
5. G. K. Hunter, K. S. Wong, and J. J. Kim, Arch. Biochem., **260**, No. 1, 161 (1988).
6. Y. Kuboki, H. Takita, T. Komori, et al., Calcif. Tiss. Int., **44**, No. 4, 269 (1989).
7. F. C. McLean and A. B. Hastings, J. Biol. Chem., **108**, 285 (1935).
8. P. A. Price, J. W. Lothringer, S. A. Baukol, and A. H. Reddi, J. Biol. Chem., **256**, 3781 (1981).
9. J. D. Andrade (ed.), Surface and Interfacial Aspects of Biomedical Polymers, Vol. 2, New York (1985).
10. J. D. Termine, H. K. Kleinman, S. W. Whitson, et al., Cell, **26**, 99 (1981).

RESPIRATION OF RAT BRAIN MITOCHONDRIA DURING HYPEROXIA AND NORMOXIA

V. A. Khazanov and A. N. Poborskii

UDC 576.311.347:51-07

KEY WORDS: mitochondria; hyperoxia; normoxia

Energy-producing reactions of mitochondria (Mch) depend on the partial pressure of O_2 and CO_2 in the medium surrounding the organelles [1, 3, 4, 9]. Traditionally the isolation of Mch and the study of their respiratory activity have been carried out in a hyperoxic medium saturated with air, in which the partial pressure of CO_2 is lower than in the intracellular medium [5]. However, the known effect of O_2 on activity of the respiratory chain, of dehydrogenases, and of other thiol enzymes of Mch [1, 2, 7, 10] suggests a considerable change in the functional state of the organelles during their isolation and study under hyperoxic conditions.

In order to determine the character of changes in respiration of Mch as the conditions of their functioning in vitro, with respect to O_2 concentration, are brought closer to those of existence in vivo, a comparative study was made of rat brain Mch on media saturated with air (hyperoxia) and saturated with a gas mixture containing O_2 in a concentration corresponding to the intracellular level (normoxia).

EXPERIMENTAL METHOD

Experiments were carried out on brain mitochondria from Wistar rats. The organelles were isolated by differential centrifugation, using methods preserving their native state [5, 7]. The isolation medium of Mch was: sucrose 0.3 M, HEPES 10^{-2} M, EDTA 10^{-4} M, pH 7.4, t = 0 ± 1°C. The composition of the incubation medium was: sucrose 0.17 M, KCl $4 \cdot 10^{-2}$ M, HEPES 10^{-2} M, KH_2PO_4 $5 \cdot 10^{-3}$ M, $KHCO_3$ $8 \cdot 10^{-3}$ M, EDTA 10^{-4} M, pH 7.4, t = 26°C. The respiratory activity of the organelles was recorded polarographically, as the rate of O_2 consumption of the Mch suspensions in different metabolic states after Chance [5]. The apparatus included a polarograph (PA-2, Czechoslovakia) and a thermostated cell of original design with membrane electrode of Clark type. The oxidation substrate was $5 \cdot 10^{-3}$ M succinate. The degree of

Research Institute of Pharmacology, Tomsk Scientific Center, Academy of Medical Sciences of the USSR. (Presented by Academician of the Academy of Medical Sciences of the USSR E. D. Gol'dberg.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 9, pp. 258-260, September, 1991. Original article submitted December 10, 1990.

 0007-4888/91/0009-1258$12.50