CALCIUM-BINDING PROPERTIES OF PLASMA PROTEINS AFTER ALLOGRAFTING OF A CADAVERIC KIDNEY

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It has been suggested that in patients after allografting of the cadaveric kidney (ACK) disturbances in the concentration of ionized calcium (Ca^{++}) and in the ratio between concentrations of total calcium and Ca^{++} in the fasting state may be caused not only by the moderate hypoalbuminemia, but also by disturbances of the calcium-binding properties of the plama proteins [2, 3]. The aim of the present investigation was to study the calcium-binding properties of the plasma proteins after ACK, in the fasting state, and in the presence of experimental hypo- and hypercalcemia. The immediate tasks of the investigation were to establish the mechanism of calcium binding by plasma proteins and to determine the quantitative parameters of binding.

EXPERIMENTAL METHOD

Altogether 60 patients aged from 15 to 48 years were studied after ACK. The control group consisted of 28 healthy individuals. The process of calcium binding by the plasma proteins was studied in experiments in vitro. For this purpose, 40-60 ml of blood was withdrawn from the cubital vein of each individual in the fasting state, without stasis, under anaerobic conditions, into syringes containing heparin (2 U/ml blood); the plasma was divided into 4-6 equal volumes and, in order to obtain a range of Ca^{++} concentrations in samples of 0.5 to 1.5 mmole/liter, depending on the original Ca^{++} concentration, 20, 40, 60, or 100 µl of a 0.125 mM solution of EDTA was added to two or three of them, and 20, 40, or 60 µl of a 90 mM solution of $CaCl_2$ was added to one or two of the volumes. From each portion of plasma, under anaerobic conditions, the ultrafiltrate was obtained in two parallel determinations in a volume not exceeding 20% of the volume of the plasma [6]. Total calcium in samples of plasma and ultrafiltrate was determined on an atom-absorption spectrophorometer (IL-151, USA), and in samples of plasma, Ca^{++} was determined on an SS-20 analyzer ("Orion Research," USA), pH an pCO_2 were determined on an AVL-940 gas analyzer (Switzerland), total protein was measured by the biuret method, and albumin by electrophoresis on cellulose acetate film.

The following parameters were calculated: the concentration of standardized Ca^{++} [4], protein-bound calcium (CaPr), the relative concentration of calcium bound with 1 mmole albumin (CaPr/A) at pH 7.4 [1]. The mechanism of calcium binding by plasma proteins was estimated in Langmuir and Scatchard plots [5]. The association constant (K_a) and the number of binding sites (n) on the protein (albumin) molecule during successive addition of calcium ions was determined on a Scatchard plot (from the coefficient of regression and X_0, respectively). In the case of a cooperative mechanism, the effective K_a and the effective n were calculated for Cu^{++} = 1 mmole/liter by the equations:

$$K_a = \frac{[CaPr/A]^2}{\beta_{sp}}, \quad n = [CaPr/A] \cdot \left(\frac{1}{K_a} + 1\right),$$

where β_{sp} denotes the specific buffer capacity of the proteins, determined from the regression coefficient in Langmuir coordinates, and CaPr/A is the experimental or calculated value, according to the regression equation for Ca^{++} = 1 mmole/liter.

These equations were obtained by transforming equations describing the kinetics of the cooperative binding mechanism:

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TABLE 1. Parameters of Binding of Calcium by Plasma Proteins after ACK (M ± m)

Binding parameters	Healthy subjects (n=20)	Group 1				
		A (n=10)	B (n=15)	C (n=2)		
CaPr/A·Ca^{++}=1	1,92±0,35	2,12±0,25	1,37±0,3*	3,46±0,028*		
β_{sp}	2,96±0,55	3,12±0,59	2,00±0,27*	4,49±0,014*		
Ca^{++} at CaPr/A = 0 (x_0)	0,33±0,2	0,30±0,12	0,31±0,13	0,23±0,2***		
K_a Ca^{++} = 1, L/mm	1,33±0,51	1,48±0,37	0,97±0,35**	2,67±0,06*		
n Ca^{++}=1	3,42±0,29	3,5±0,25	2,92±0,21*	4,76±0,014*		
			Group 2			
	(n=13)	(n=6)	(n=11)	(n=4)	(n=11)	
CaPr/A·Ca^{++}=1	1,84±0,24	1,86±0,12	1,84±0,24	1,19±0,38*	3,04±1,66	
β_{sp}·Ca^{++}=1	1,45±0,21	1,51±0,12	0,50±0,30*	0,81±0,29*	2,02±0,93	
K_a, L/mm	0,28±0,22	0,24±0,15	6,15±7,76*	1,14±2,27	1,34±2,35	
n	12,7±6,7	13,0±6,55	2,70±0,59*	4,94±3,13	22,6±17,2	

Note. *p < 0.01, **p < 0.02, ***p < 0.05 compared with healthy subjects. Number of observations given in parentheses.

$$[\mathrm{CaPr/A}] = \frac{K_a \cdot I_{max} \cdot [Ca^{++}]^n}{1 + K_a \cdot [Ca^{++}]^n}, \quad (1)$$

$$\beta_{sp} = \frac{K_a \cdot I_{max}^2 \cdot [Ca^{++}]^{n-1}}{(1 + K_a \cdot [Ca^{++}]^n)^2} \quad (2)$$

on the assumption that n for Ca^{++} = 1 mmole/liter approximates to n_{max}. This assumption is based on experimental data obtained on healthy individuals relating to completion of the cooperative mechanism of binding at Ca^{++} = 1.01 ± 0.06 mmole/liter.

With noncooperative binding β_{sp} and CaPr/A for Ca^{++} = 1 mmole/liter were calculated on the basis of values obtained for K_a and n:

$$\beta_{sp} = \frac{K_a \cdot n}{(1 + K_a)^2}; \quad \mathrm{CaPr/A} = \frac{K_a n}{1 + K_a}.$$

EXPERIMENTAL RESULTS

The cooperative mechanism of calcium binding by proteins was established in 33 patients after ACK: a positive value of X in Lnagmuir coordinates (Table 1) and positive correlation in Scatchard coordinates. In 27 of them a cooperative binding mechanism was observed with the same Ca^{++} concentrations in the plasma as in healthy individuals (Group 1), and in five the mechanism was complete at Ca^{++} below 0.9 mmole/liter. According to the value of CaPr/A at Ca^{++} = 1 mmole/liter, reflecting affinity of the proteins for calcium, the patients of Group 1 were divided into subgroups: A) with normal, B) with reduced, and C) with increased affinity (Table 1). In Group 1A the binding parameters were the same as in healthy subjects. In Group 1B reduction of CaPr/A was associated with a decrease in β_{sp}, and was caused by a decrease in the value of effective K_a and effective number n on the protein molecule at Ca^{++} = 1 mmole/liter. In one patient (not included in Table 1) a sharp reduction of CaPr/A at Ca^{++} = 1 mmole/liter was combined with normal β_{sp} and was caused by a marked decrease of K_a to 0.01 L/mm and an increase in n to 21, which was manifested as a marked shift of the association curve to the right (X_0 = 0.93 mmole/liter). In Group 1C the increase in CaPr/A was associated with an increase in β_{sp} and was caused by an increase in all the binding parameters, manifested as a shift of the association curve to the left. In 32 patients (Group 2) successive addition of calcium to one or two sets of binding sites on the protein molecule was established: a negative value of X_0 at Ca^{++} = 1 mmole/liter in Langmuir coordinates and negative correlation in Scatchard coordinates (five patients in whom consecutive addition of calcium to the binding sites on protein did not begin until Ca^{++} > 0.9 mmole/liter, and at higher Ca^{++} concentrations a cooperative mechanism of binding was discovered, also were included in the group). Depending on the value of CaPr/A at Ca^{++} = 1 mmole/liter, patients of Group 2, like those of Group 1, were divided into subgroups: A) with normal, B) with reduced, and C) with increased affinity of the plasma proteins for calcium (Table 1). In six patients in Group 2A all binding parameters were the same as in healthy subjects with normo- and hypercalcemia, while in 11 patients a normal value of CaPr/A was combined with a low value of β_{sp}, as a result of reduction of n and a considerable increase in K_a. In group 2B a decrease in CaPr/A was associated with a decrease in β_{sp}, which appeared as a result of

reduction of n and a tendency for K_a to increase. In Group 2C increased affinity of the proteins for calcium was acompanied by only a tendency for β_{sp}, K_a, and n to increase, possibly due to the small size of the group.

Thus in 5/6 of the patients after ACK different disturbances of calcium-binding properties of plasma proteins were discovered. In one-third of them the abiity of the proteins to bind calcium cooperatively was preserved, but the binding parameters underwent changes which led in most patients to a decrease of affinity (reduction of β_{sp}, CaPr/A, K_a, and n), but in two patients, to increased affinity (an increase in β_{sp}, CaPr/A, K_a, and n) for calcium. In more than half of the patients the properties of the plasma proteins were grossly disturbed, as was shown by loss of their ability to carry out cooperative calcium binding in hypocalcemia. In two-thirds of them, reduction of β_{sp} of the proteins was found, as a result mainly of a decrease in the number of calcium binding sites, combined in one-third of cases with a fall of CaPr/A. Only in four patients was CaPr/A increased. The present investigation confirms the previous hypothesis regarding disturbance of the calcium-binding properties of the plasma proteins after ACK, and is evidence of yet another manifestation of disturbances of protein metabolism, leading to a disturbance of homeostasis for ionized calcium.

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