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Inhibition of transport of ^{47}Ca and ^{85}Sr by lanthanum in canine cortical bone

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Abstract

Deposition of ^{85}Sr and ^{47}Ca and blood flow (measured by iodoantipyrine washout) were determined in the tibial cortex of adult dogs after injection of graded doses of lanthanum chloride (LaCl_3) and potassium cyanide (KCN) into the right tibial nutrient artery. Deposition of ^{85}Sr and ^{47}Ca , expressed in milliliters per gram of cortical bone in 10 min, was decreased after injections of lanthanum, 0.045 ± 0.008 (mean $\pm$ SE) compared to 0.097 ± 0.01 in control experiments ($P < 0.005$). Blood flow was unchanged. Injection of KCN did not affect the mean value of uptake of mineral (0.108 ± 0.01 vs. 0.097 ± 0.01) over the whole range of KCN dosage. Blood flow tended to be slightly higher with lower doses of KCN. These data support the concept of a transport system in bone for bone-seeking isotopes such as ^{85}Sr and ^{47}Ca .

Keywords

deposition of ^{85}Sr and ^{47}Ca ; mineral transport; KCN; bone blood flow; transport system

A critical issue in physiology of bone is whether or not ion concentrations in bone are regulated by a functional membrane that separates extracellular fluid of bone from extracellular fluid of blood. The evidence for the existence of a functional membrane in bone is based in part on in vitro experiments (9,21) and in part on hypothesis (17,18,23). Ramp and Neuman (21) found that both metabolic poisoning of tissue cultures of tibias from Leghorn chick embryos and splitting of the embryonic bone increased the mineral content of the bone. These data were interpreted to indicate that disruption of intact bone led to loss of membrane compartmentalization and allowed accumulation of mineral in bone. Geisler and Neuman's (9) observation of selective concentration of potassium in bone also suggests membrane activity. The membrane in bone is not well defined. Talmage (23) has considered it to be a continuous layer of cells composed of active and resting osteoblasts and the cells lining the vascular channels of bone, while Neuman and Ramp (17) expressed the opinion that membrane compartmentalization in long bone is effected by periosteum, endosteum, and the walls of the blood vessels in the bone.

If there is a functional membrane in bone, it seems likely that a carrier would exist for transport of minerals that concentrate in bone. For example, Lehninger (14) has provided evidence for Ca^{2+} carrier transport in mitochondria, and others have observed carrier-type kinetics in erythrocytes, squid nerve axon, kidney tubules, and muscle cells, some of which have been reviewed by Bassingthwaighe and Reuter (1). In agreement with Williams' (26) suggestion, based on theory, that a phosphorylated protein may serve as a carrier, Wasserman et al. (24) have described a calcium-binding protein for calcium transport across the intestinal epithelium, and MacLennan and Wong (15) have identified, in the sarcoplasmic reticulum of rabbit skeletal

muscle, a phosphorylated Ca^{2+} transporting protein (an ATPase) and a nonphosphorylated binding or sequestering protein.

Lanthanides may compete for Ca^{2+} and Sr^{2+} binding sites on the cell membrane (8) or on a carrier protein (16). Williams (26) pointed out that the similarity in size of this trivalent ion to that of Ca^{2+} is the main basis for its ability to substitute for Ca^{2+} at binding sites. Experimentally, Mela's (16) data on Ca^{2+} uptake in liver mitochondria suggest that La^{3+} blocks a phosphorylated carrier. Blockage of a carrier by La^{3+} implies that it binds to the transport site more strongly than do Sr^{2+} and Ca^{2+} and also suggests that the carrier may not transport La^{3+} across the membrane, because of its larger size. Reuter (22) showed and reviewed evidence that La^{3+} blocks Ca^{2+} conductance in cardiac muscle, presumably by attaching to the transport site.

This study was performed to determine if La^{3+} (as LaCl_3) will alter deposition of ^{85}Sr and ^{47}Ca in bone. One possibility is that La^{3+} might cause an increased deposition of ^{45}Ca or ^{85}Sr if it blocked efflux from bone. This probably would not be evident in the early stages of uptake of tracer because the tracer is diluted so greatly in the large calcium pool of bone (3). Inhibition of tracer entry into bone seems more likely, because blockage of facilitating transport sites, whether they be on the capillary membrane or on a specialized bone membrane, would inhibit transport unless there are large, readily permeated, passive diffusion channels that would effectively bypass the cells. A subsidiary aspect was to determine if graded doses of KCN affect deposition of ^{85}Sr and ^{47}Ca ions, an effect that might occur if the transport system were tightly coupled to energy sources dependent on the hydrogen acceptor system.

METHODS

The 49 dogs were anesthetized and heparinized (2.5 mg/kg of body wt). The popliteal artery and vein, anterior tibial artery and vein, and origin of the nutrient artery and vein were exposed by removal of the fibula. The deep digital flexor was retracted and, by careful dissection, the nutrient artery and vein were identified in the nutrient canal; the artery was cannulated. The fascia overlying the flexor muscle was closed with interrupted sutures and the skin was closed with a running suture (11).

In the first group (10 dogs), after cannulation of the right tibial nutrient artery, cortical bone blood flow was estimated by [^{125}I]iodoantipyrine (IAP) washout (13). Then, a slug injection of LaCl_3 was given in dosages ranging from 0.04 to 5.5 mg/g of tibia. Following this, cortical bone blood flow was again determined by IAP washout. Thirty minutes later, after counts had returned to background value, a similar dose of LaCl_3 was injected and then uptake of either ^{85}Sr or ^{47}Ca was determined by a modification of a method previously described (25).

In the second group (29 dogs), the same protocol was followed but instead of LaCl_3 , KCN was injected in doses ranging from 0.0006 to 0.83 mg/g of tibia.

In the third group of experiments (10 dogs), the same protocol was followed except that only the diluent was injected, free of either KCN or LaCl_3 .

Determination of cortical bone blood flow

The details and validation of the method have been published (13).

Fifteen microcuries of IAP were dissolved in 0.5–1 ml of isotonic saline and injected rapidly into the tibial nutrient artery. The isotope emission curves, $C^*(t)$, were recorded on a strip recorder from an analog rate meter. The flow, f' (ml/ml bone per min), was estimated by the equation:

$$f' = \lambda \cdot \frac{C^*(0) - C^*(T)}{\int_{t_0}^T C^*(t) dt} = \frac{1 - R(T)}{\int_{t_0}^T R(t) dt}$$

in which $R(t)$ is the fraction of the injectate remaining in the bone and can be considered to be $C^*(t)/C^*(0)$. $C^*(t)$ is the count rate at time, t , measured by the external detector, a sodium iodide (thallium-activated) crystal 1.9 cm in diameter and thickness, shielded with a 9.5-mm layer of brass and lead and placed over the anterolateral middiaphysal surface of the tibia. The pulse-height window settings were selected to detect the gamma energy between 0.0175 and 0.050 MeV. $C^*(0)$ is the peak activity and occurs at $t = 0$, which is a few seconds after the injection at t_0 . The time, T , of ending the integration of the area was arbitrarily chosen to be 15 min because the estimate of flow is not significantly changed by using a longer period (13). Bone specific gravity, ρ , is taken to be 1.96 and the bone-blood partition coefficient, λ , to be 0.15, as previously reported (13).

Determination of ^{47}Ca and ^{85}Sr deposition

Carrier-free solutions of ^{47}Ca and ^{85}Sr were obtained from Abbott Laboratories (North Chicago, IL). These radioisotopes were diluted with saline to provide approximately 20 μCi /ml of solution. The gamma peaks are 0.51 MeV for ^{85}Sr and 1.30 MeV for ^{47}Ca . The half-lives are 64 days for ^{85}Sr and 4.7 days for ^{47}Ca . ^{47}Se , the breakdown product of ^{47}Ca , has a gamma peak of 0.16 MeV and was eliminated from ^{47}Ca and ^{85}Sr counting by adjusting the pulse-height analyzer to exclude this radiation. Counting was performed in a well-type scintillation counter (Nuclear-Chicago, Des Plaines, IL) for 2 min or more.

The right jugular vein and left carotid artery were cannulated with plastic cannulas (0.070 mm ID, 0.110 mm OD). Via the cannula in the vein, each dog was injected with 10 μCi of ^{85}Sr or ^{47}Ca and the isotope was flushed through the cannula with isotonic saline. At 1-min intervals after isotope injection, 2-ml arterial blood samples were drawn from the left carotid cannula. After the 10-min sample was drawn, the dog was killed by an intravenous injection of 300 mg of pentobarbital. Both tibiae were rapidly disarticulated and cleaned of soft tissue. Then, a cross-sectional segment, approximately 1.5 cm in width and corresponding to the area scanned by the external detector, was removed, cleaned of marrow, and weighed; the counts, B counts/s per g of bone, were obtained. Arterial blood samples were counted in the same apparatus and the average arterial blood activity over the initial 10-min period was determined by measuring the resultant curve with a planimeter and dividing by 10 to calculate the average inflow concentration, A counts/s per milliliter of blood. Tracer deposition was calculated as follows:

$$\text{tracer deposition (ml/g) at 10 min} = \frac{A \text{ counts/s per g bone}}{B \text{ counts/s per ml blood}}$$

Figure 1 represents a typical ^{85}Sr blood disappearance curve from a control dog. The choice of the 10-min period is based on autoradiographic data which showed that ^{45}Ca was mainly concentrated on bone surfaces where blood vessels are known to traverse (haversian canals, endosteal and periosteal surfaces, and resorption spaces [19]). Exchange during this first 10 min represents an initial bone clearance of ^{85}Sr and is directly dependent on effective blood flow to bone (6).

RESULTS

The injection of LaCl_3 decreased uptake of ^{85}Sr and ^{47}Ca on the right side (test side) when compared to the control dogs or to the left side of the same dogs ($P < 0.005$) but did not alter blood flow as measured by IAP washout (Table 1).

Figure 2 (lower) shows that La^{3+} reduced uptake to an average of 38 % of the control. The suggestion of greater inhibition at higher doses was not statistically significant. Effects on flow were random (Fig. 2, upper).

The effects of KCN are shown in Fig. 3. Both flow and mineral deposition were slightly increased at low doses (0.006–0.08 mg/g of tibia), and both were decreased at high doses (0.21–0.83 mg/g of tibia). The mean values for deposition and flow over the whole range of KCN dosages were the same on test and control sides (Table 1).

DISCUSSION

The present study shows that LaCl_3 decreases deposition of ^{85}Sr and ^{47}Ca in cortical bone. This phenomenon is not an effect of LaCl_3 on tibial blood flow because blood flow was unchanged.

The prevailing theory regarding calcium transport in bone as proposed by Talmage (23) suggests that the two fluid compartments in bone are separated by a continuous layer of cells, the family of osteoblasts and the cells lining vascular channels. One fluid compartment, the bone extracellular fluid, is directly associated with mineral; the other fluid compartment is the extracellular fluid of the blood. It is further assumed that the calcium concentration is different on the two sides of the membrane and that calcium is continuously transported outward against the gradient (away from bone). Transcellular calcium transport is accomplished by permitting calcium to come in with the gradient and pumping it out by a specific calcium transport system on the other side of the same cell. The findings of Ramp and Neuman (21) *in vitro*, that metabolic poisoning results in accumulation of mineral, support this hypothesis. In gut and kidney, epithelial junctures are tight and calcium movement is in one direction—from lumen into blood through cells. In bone, the channels between the cells are not as tight, and it is supposed that this will allow easier movement of mineral across the hypothetical membrane into the bone crystal structure.

It would appear that La^{3+} does not interfere only with active efflux of calcium from bone to blood because, if it did, deposition of ^{85}Sr and ^{47}Ca would be increased. At low doses KCN might be blocking the extrusion process, but the data are not conclusive. The ratios of values in the lower panel of Fig. 3 to those in the upper panel (mineral deposition, test/control divided by flow, test/control) averaged 1.7 (excluding one high value) but were not systematically influenced by the dose of KCN; the value greater than 1 suggests that mineral deposition is somewhat increased relative to the flow.

The mechanisms for Ca^{2+} influx from blood to bone include transcellular transport and diffusion through extracellular space. Baud (2) has demonstrated open channels of 400 Å or greater between cells lining bone, and, if the total area of these were large, passive diffusion would dominate the influx to bone. But the inhibition by La^{3+} shows that a transmembrane process is involved. (The diffusion of Ca^{2+} or Sr^{2+} can be increased in the presence of La^{3+} , since La^{3+} will occupy binding sites that would otherwise slow Ca^{2+} movement, but La^{3+} cannot decrease the diffusion coefficient.) The nature and location of the transcellular transport process cannot be precisely defined by these experiments, but, because of Baud's observations on the wide separations between osteoblasts, osteoclasts, and osteocytes, these cells are likely to be bypassed by diffusion and therefore unlikely to be involved in the transport. Thus, as

Neuman and Ramp (17) suggested, the transporting membranes may be endosteum, periosteum, or endothelium of cortical capillaries. (Capillary membranes in the brain have been shown, by Crone and Thompson (7), to have a carrier-mediated transport mechanism for glucose.) If a carrier does exist, then it is possible that La^{3+} takes the place of ^{85}Sr or ^{47}Ca on the carrier, and deposition of these ions is decreased. What this carrier might be is unknown but it is conceivable that it is a glycoprotein (sialoprotein) (10,14,20).

Mineral deposition values expressed as milliliters/gram of bone for 10 min become clearance values if divided by the duration of the experiment. Furthermore, if IAP flow is expressed as milliliters/gram of bone, mineral deposition and IAP flow values should be comparable. For example, in the control group the mineral deposition was 0.0097 ml/g bone per min compared to IAP flow of 0.0088 ml/g bone per min. This does not imply that clearance is higher than flow; the value 0.0088 is for cortical bone and does not include the one-third of the nutrient artery flow that supplies the marrow (12). Bosch (3) found only 0.4 % of administered ^{85}Sr in marrow, and the implication is that the ^{85}Sr that enters via marrow circulation is cleared by tibial cortex. This is in agreement with Brookes and associates' (4) anatomic studies suggesting that nutrient artery flow enters endosteally and exits periosteally.

The present experiments with KCN suggest that this transport system and also the deposition of mineral in bone do not appear to be strongly coupled to an intracellular oxidative process. However, one cannot rule out the possibility of a transient metabolic bypass, such as the anaerobic glycolysis demonstrated in epiphyseal-metaphyseal bone slices (5), which could temporarily compensate for the toxic effect of KCN.

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FIG. 1.
Disappearance curve for ^{85}Sr from arterial blood sampled at 1-min intervals.

FIG. 2.

Effect of La^{3+} on blood flow (*upper*) and mineral deposition (*lower*). Test side was right tibia; control side was left.¹

¹For tables of individual data points, order NAPS Document 02298 from Microfiche Publications, 305 East 46th St., New York, N.Y. 10017, remitting \$1.50 for microfiche or \$5.00 for photocopies.

FIG. 3.

Effect of KCN on blood flow (*upper*) and mineral deposition (*lower*). Correlation coefficient, r , for log deposition ratio versus dose is -0.53 ($P < 0.005$). For log flow ratio versus dose, $r = -0.62$ ($P < 0.001$).¹

TABLE 1Effect of LaCl_3 and KCN on mineral deposition and blood flow in bone

<i>N</i>	Mineral Deposition, ml/g bone in 10 min		Blood Flow, IAP Method, ml/ml bone per min	
	Test	Control	Before test	After test
LaCl ₃	10	0.0446 ± 0.008	0.1234 ± 0.0200	0.0121 ± 0.0010
KCN	29	0.108 ± 0.0100	0.107 ± 0.0100	0.0171 ± 0.0010
Control	10	0.097 ± 0.0100	0.0170 ± 0.0020	0.0150 ± 0.0020