Cation Exchange Properties of Bone Tissue

A very high uptake of ^{51}Cr-β-glycerophosphate by bone tissue has been noticed when the biological behaviour of this proposed tumor localizing agent[1] was studied. The Table shows the values of a typical radioactivity distribution in rats 24 h after i.v. injection. The concentration of radioactivity by the bone does not agree with its glycerophosphate hydrolytic capability (phosphomonoesterase activity). The possibility of a simple cation exchange was assayed by in vitro incubation of bone tissue with ^{51}Cr-β-glycerophosphate.

A duplicate assay was performed with 2 rat femurs from the same animal. One of them was autoclaved 20 min in order to destroy any enzymatic activity. After this, it was ground and washed 5 times with distilled water in order to eliminate all the hydrolyzed proteins. The other femur kept at 4 °C was also ground. 1 g of each was suspended in 5 ml of saline plus 5 ml of lactated Ringer's solution (Hartmann's). After addition of 0.5 ml of ^{51}Cr-β-glycerophosphate complex (2.4 mg Cr/ml), they were incubated for 1 h at 37 °C with occasional stirring. After incubation, they were centrifuged and the residue washed 5 times with normal saline. The radioactivity was measured in both residues and supernatants with a scintillometer. 33.9 and 22.4% of the total radioactivity remained in the residue of preheated and normal femur respectively. The higher exchange with the heated bone can be explained as a consequence of the increase in the active surface of the mineral tissue due to the hydrolysis of the collagen.

Radioactivity distribution after i.v. injection of ^{51}Cr-β-glycerophosphate into rats

% dose/g				
Blood	Liver	Spleen	Kidney	Femur[a]
0.02	0.08	0.10	0.48	0.64

[a] Ratio (radioactivity mineral tissue/radioactivity bone marrow) per gram = 20.

In another experiment, the exchange was tested with carbonates and phosphates similar to the mineral part of the bone tissue. To a suspension of 100 mg of $CaCO_3$, $MgCO_3$, $SrCO_3$, $Mg_3(PO_4)_2$ and $Ca_3(PO_4)_2$ in 5 ml of distilled water (buffered to pH 7.2 with KH_2PO_4), 0.2 ml of ^{51}Cr-β-glycerophosphate (8.1 mg Cr/ml) were added and incubated at 37 °C for 1 h. After centrifugation and washing, the radioactivity of both residues and supernatants was counted as described before. The percentages of the used radioactivity remaining in the insoluble (exchanged) were: $CaCO_3 = 4.3$; $MgCO_3 = 81.0$; $SrCO_3 = 5.1$; $Ca_3(PO_4)_2 = 30.4$ and $Mg_3(PO_4)_2 = 87.0$.

These experimental findings indicate that through a simple cation exchange mechanism some of the mineral constituents of bone are able to deposit as an insoluble compound the chromium from a very stable β-glycerophosphate complex.

Riassunto. È presentato uno studio sulla fissazione del ^{51}Cr-β-glicerofosfato. Il ^{51}Cr di questo composto qui presenta una elevata stabilità chimica è fissato nella parte minerale dell'osso per un meccanismo d'intercambio cationico.

L. J. ANGHILERI[2]

The Johns Hopkins Medical Institutions, Department of Radiological Science, Baltimore (Maryland 21205, USA), 11 October 1968.

[1] L. J. ANGHILERI, Oncology *21*, 275 (1967).

[2] Actual address: Div. Nuclear Medicine, University of Colorado, Medical Center, Denver, Colorado.

Pharmacological Studies on Thiamine Deficiency IV. Blood Catecholamine Content and Blood Pressure of Thiamine Deficient Rats

As shown in previous reports from this laboratory, the catecholamine (CA) contents in the brain cortex, in the heart atria and ventricles and in the spleen are significantly increased in thiamine deficient rats as compared with those of thiamine supplemented or pair-fed control group[1] and impairment of tissue monoamine oxidase (MAO) activities plays some role in the CA accumulation[2]. Furthermore, this increase of CA concentration may result in changes in some pharmacological responses[1,3].

In this work, to test whether changes in the spontaneous release of CA from the tissues were involved in the accumulation in deficient animals, the CA concentration in the blood was measured with special reference to its relation to change in blood pressure. In addition, the pressor effects of DL-noradrenaline (NA) and tyramine in thiamine deficient rats were examined.

Materials and methods. Using male Sprague-Dawley rats, weighing 80–100 g, as experimental animals, a thiamine deficient group, a pair-fed and a control groups were prepared and a decision for the deficiency of the animal was done as described previously[1,2].

The blood CA content of rats was measured by a modification of the method of CROUT[4]: animals were sacrificed by decapitation and blood from the carotid arteries was taken into a tube containing ice-cold 0.4 N perchloric acid. The tube was kept at -20 °C until the following day and then thawed. In addition to the original procedure for elution with alumina, Dowex 1×8 column was also employed[5].

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Results and discussion. The Table shows that the CA concentration in the blood of thiamine deficient rats was about one-half of that in either the control or pair-fed group, while there was no difference between the contents of the control and pair-fed groups. Lowering the blood CA concentration might result from decreased spontaneous release of the amine from tissues in thiamine deficient rats, so this seems to be one factor causing accumulation of CA in thiamine deficient rat tissues.

Blood catecholamine content of thiamine deficient rats

	Catecholamine content (μg/ml)	P [c]
Control [a]	0.030 ± 0.002 (5)	< 0.01
Thiamine deficient	0.015 ± 0.001 (4)	
Pair-fed [b]	0.025 ± 0.003 (4)	< 0.05

[a] Fed on the thiamine deficient basal diet supplemented with 3 mg thiamine hydrochloride per kg. [b] Given daily an amount of control diet of the same weight as that eaten by the deficient group. [c] Significant P values in relation to the thiamine deficient group. Four rats were used in each experiment and the values represent the mean ± S.E. of the number of experiments shown in parenthesis.

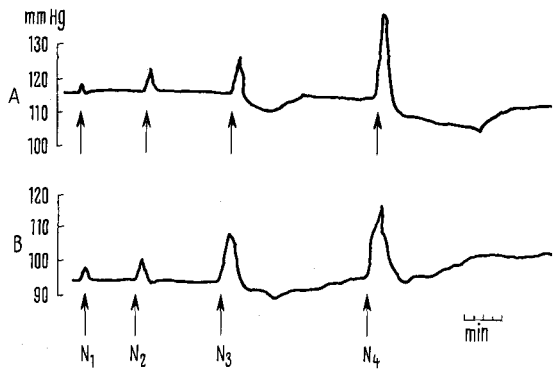

Fig. 1. Blood pressure of carotid artery of thiamine deficient rat and effect of DL-noradrenaline on it. N_1, N_2, N_3, N_4 (DL-noradrenaline hydrochloride 0.5, 1.0, 2.5, 5.0 μg/kg, respectively). (A) Control rat, (B) thiamine deficient rat. Anesthesia was done with 1.2 g/kg i.p. of urethane. Drugs were given into femoral vein.

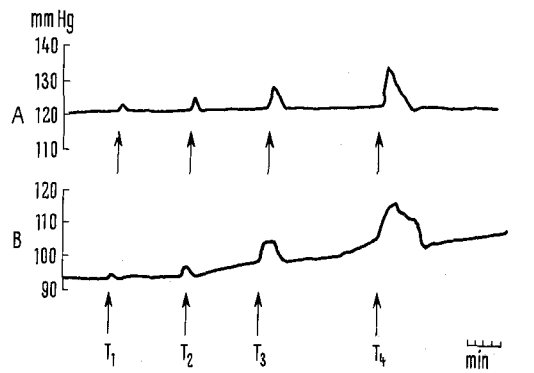

Fig. 2. Blood pressure of carotid artery of thiamine deficient rat and effect of tyramine on it. T_1, T_2, T_3, T_4 (tyramine monohydrochloride 0.1, 0.2, 0.5, 1.0 mg/kg, respectively). Other conditions are the same as in Figure 1.

Further experiments on this, such as those on CA uptake are required to understand the overall mechanism clearly. The correlation between the inhibition of amine release and the impairment of MAO activity is still obscure. However, with regard to this, the findings of AXELROD[6] should be kept in mind. They showed that the release of CA was inhibited by MAO inhibitors.

We studied the pressor effects of NA and tyramine in thiamine deficient rats next. 10 of 14 deficient animals showed marked hypotension, ranging from 86–100 mm Hg, relative to the control group (113–126 mm Hg). Two examples are shown in Figures 1B and 2B. From this result, it seems that hypotension in thiamine deficient rats may be due to lowering of the blood CA concentration. In relation to this, it is also possible that sensitivity to this transmitter could be reduced in thiamine deficient animals. To examine this possibility, the pressor response to NA was determined. As shown in Figure 1, the increase in blood pressure evoked by NA injection was similar in the thiamine deficient and control groups. Moreover, no difference in the pressor response to tyramine in these 2 groups could be found (Figure 2). This was, however, unexpected, because in our previous publications the following findings had been reported: (a) the effect of tyramine on an excised heart from a deficient rat is much more than that on the heart of a control animal[1], and (b) the CA release after tyramine injection in deficient animals is greater than that in the control group and is accompanied by a marked raise in heart rate[3]. However, it must be also taken into consideration that changes in systemic blood pressure by drugs are manifested in general as the sum of the effects of various factors.

On the other hand, the blood pressure in the thiamine deficient group gradually increased with successive injections of tyramine. This result suggests that the elevation of the basal blood pressure did not result from a direct action of tyramine itself, and that the so called available CA[7] plays some role in regulating blood pressure, when taken into consideration our previous finding[3].

To summarize the evidence presented so far, one could say that owing to the lowering of blood CA concentration in the thiamine deficient rat, the animal usually becomes hypotensive. Reduction of spontaneous release of CA from tissues containing amine in thiamine deficient rats is thought to be one factor contributing to CA accumulation in the deficiency.

Zusammenfassung. Wegen der Senkung des Blut-Katecholamin-Spiegels in unter experimentell erzeugtem Thiaminmangel stehenden Ratten kommt es zu einer Hypotonie. Die Abschwächung der spontanen Freisetzung des Katecholamins aus dem katecholaminspeichernden Gewebe bei Thiaminmangelratten ist als ein Faktor bei der Aminakkumulation bei Thiaminmangel zu denken.

H. IWATA, T. NISHIKAWA
and K. WATANABE

Department of Pharmacology,
Faculty of Pharmaceutical Sciences, Osaka University,
Toneyama, Toyonaka, Osaka-fu (Japan),
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