East African Medical Journal Vol. 79 No. 4 April 2002
EFFECTS OF HYPOKINESIA ON CYCLIC NUCLEOTIDES AND HORMONAL REGULATION OF CALCIUM METABOLISM IN RATS
J.B.L Chek, MSc, PhD, Senior Lecturer, Department of Biochemistry, College of Biological and Physical Sciences, University of Nairobi, P.O. Box 30197, Nairobi, Kenya and A.I. Laniko, MSc, PhD, Professor of Biochemistry, Kharkov State University, Ukraine Republic.

Request for reprints to: Dr. J.B. L. Check, Department of Biochemistry, College of Biological and Physical Sciences, University of Nairobi, P O.Box 30197, Nairobi, Kenya

EFFECTS OF HYPOKINESIA ON CYCLIC NUCLEOTIDES AND HORMONAL REGULATION OF CALCIUM METABOLISM IN RATS

J.B.L CHEK and A.I. LANIKO

ABSTRACT

Objective: **To study the dynamic of changes in the level of parathyroid hormone (PTH), calcitonin (CT), cyclic nucleotides (cAMP, cGMP) and calcium in the blood of rats, while in urine - phosphate, calcium and cyclic nucleotides**

Design: **Laboratory based experiment.**

Setting: **Laboratory in the Department of Biochemistry, Faculty of Medicine, University of Nairobi, Nairobi, Kenya and in the Department of Biochemistry, Kharkov State University, Ukrain Republic.**

Results: **Correlation between the changes in the parameters of study was shown. This supports the theory about the relationship between branches of hormonal systems (cyclic nucleotides - calcium), which perform the central role in the regulation of homeostasis. The results show that daily excretion of calcium and phosphate is age dependent in rats during the pathogenesis of hypokinesia: the values are higher in control groups. Changes in calcium in the blood and phosphaturia in the experimental animals of all ages were attributed to changes in the intensive re-absorption process within the bones during the readaptation period of hypokinesia.**

Conclusion: **Old rats have a higher ability to adaptation than younger experimental counterparts during hypokinesia.**

INTRODUCTION

Problems of stress and mechanism of adaptation to the effects of environmental conditions are some of the modern objectives of biology and medicine. This is caused by the increasing number and varieties of the causative factors, and by the established facts about the development of pathological changes within the vital parts of the living organism under the influence of such factors(1-3). Pathogenesis of hypokinesia is devoted attention because it is a critical situation for astronauts, and also during automation of work and mechanization in many areas of national production. These result in reduction and limitation of human movements. One of the important areas in the pathogenesis of hypokinesia involves changes in calcium homeostasis. This condition affects the functions of the whole living system, e.g. in early 1980s the role of altered Ca2+ regulation, especially in the Alzheimer's disease (AD) was formerly proposed. Over the last 50 years several variations and further modifications of this concept, involving different regulatory and pathogenic aspects of Ca homeostasis, also have been suggested(4-9). Also affected is the bone apparatus (metabolism, demineralization, reduced strength, and osteoporosis). Currently special emphasis is laid on the problems of regulating calcium metabolism in view of its role in supporting important life processes, and also in line with the theory about the role of calcium in the realization of specialized signals, in which phylogenetically it falls more ancient than hormones and cyclic nucleotides. Since intracellular free Ca2+ is a major second messenger molecule, elevated calcium concentration promotes cell survival by activating a signal transduction pathway, most likely the same way trophic factors affect survival(10,11).

It has been reported that the active mechanism of regulating calcium homeostasis requires the hormones like calcitonin (CT), parathyroid hormone (PTH) and vitamin D. The kidney and the liver are involved in this mechanism of regulating calcium homeostasis. Also involved is the system of nucleotides which act as the second messengers in the mobilization of the intracellular calcium reserves, its adaptation at a higher level of cellular response to external stimuli(12-14). However, the mechanisms related to the action of osteotrophic hormones, cyclic nucleotides and calcium in the system of regulating metabolism and functional reactivity of the bones remain unclear even under normal conditions. This study dealt with changes in the levels of PTH, CT, cyclic nucleotides (cAMP and cGMP) and calcium in the blood. Phosphate, calcium and cyclic nucleotides were studied in urine.

MATERIALS AND METHODS

Laboratory animals: Rats of wister type of different sexes, aged 3 and 12 months were used through out this study. They were subjected to hypokinesia for 7 and 14 days. Hypokinesia was conducted by employing special containers in which experimental animals were kept completely immobile. The necessary parameters were studied after the 1st, 3rd, 5th, 7th and 9th days of stopping hypokinesia

Data collection: PTH and CT levels were determined using radioimmunological methods as described in the standard kits: RIA-mat calcitonin from "BIC" Mallingrogt (Germany), and - RIA-100 from "IRE" (Belgium). The concentration of cAMP and cGMP in urine and plasma were measured using kits from

"Becton Dickson". (U.S.A.) as described by Steiner and colleagues(15). Calcium levels in the blood were measured using the method of N.L Docktorovich(16), while urine phosphorus and creatinine were respectively measured using the method of G.G Tauccki and E. Shop and Bosnes(19,20). The concentration of uronic acid carbasone (UAC) was measured using the method of Bitter and Muir(19) while Sialic acid was measured by Gess method(20).

Statistical analysis: All the data were analysed using Fisher - student in a microcomputer B3-21M.

RESULTS

Tables 1 - 3 shows the correlation between the parameters of study. This is in accordance with the theory about the relationship between branches of hormonal systems - cyclic nucleotides - calcium that occupies the central position in the regulation of homeostasis. We also managed to establish the precise difference in response to hypokinesia.

As depicted in Table 1, daily excretion of calcium and phosphate unevenly changed during hypokinesia for 7 and 14 days respectively. However, the amount of excreted phosphate did not change on the 7th day of hypokinesia. Probably during the 14 days of hypokinesia the tubular re-absorption of phosphate managed to undergo a higher degree of re-adaptation than during a short-term effect of hypokinesia. The excretion of calcium after 14 days of hypokinesia occurred more intensively than after 7 days of hypokinesia.

Table 1

Dynamic of creatinine excretion, inorganic phosphate and calcium levels in urine of 12 month old rats after 7 and 14 days hypokinesia

7 days hypokinesia Duration After hypokinesia & control	No.	Creatinine µmol/l g day	No.	phosphate µmol/l g day	No.	Calcium µg/day
Control 2	22	11.1 ± 0.1	19	16.3 ± 1.0	14	2.3 ± 0.1
Ist day	5	13.0 ± 0.1*	5	27.6 ± 2.4***	18	4.0 ± 0.3*
3rd	5	117 ± 0.1****	4	22.6 ± 2.5**	4	4.6 ± 0.1*
5th	5	10.1 ± 0.1***	7	31.5 ± 2.6*	5	4.3 ± 0.5*
7th	8	126 ± 0.3***	8	28.3 ± 1.7*	9	3.5 ± 0.4*
9th	3	13.2 ± 0.4**	3	23.6 ± 2.6*	5	3.9 ± 0.5*
14 days hypokinesia						
Control 2	22	9.4 ± 0.1	33	18.3 ± 0.8	14	2.3 ± 0.1
1st day	5	16.9 ± 0.1*	8	16.9 ± 1.7	5	4.9 ± 0.7*
3rd	5	15.8 ± 0.2*	7	21.3 ± 1.7***	6	5.1 ± 0.4
5th	9	10.9 ±0.1 ***	7	18.2 ± 0.7**	13	3.0 ± 0.3**
7th	7	18.9 ± 0.2*	6	27.7 ± 3.3*	6	4.3 ± 0.5*
9th	4	11.4 ± 0.2***	4	15.7 ± 2.5***	4	5.8 ± 0.6*

* p<0.01. ** P<0.02; *** p<0.05; ****P< 0.05 (when compared with values of control group)

Table 2

Dynamic of changes in the concentration of parathyroid hormone (PTH) calcium (CT) and calcium in blood plasma of 12 -month old rats following 7 - and 14 - days hypokinesia.

7 - days hypokinesia Duration after hypokinesia and control	No.	PTH MME/ml	No.	CT ng/ml	No.	Calcium mg/100 ml
Control	12	9.90 ± 0.45 100.0 ± 4.5%	8	34.6 ± 3.6 100.0 ± 9.5%	15	15.9 ± 0.6
1st day	5	5.90 ± 0.6 59.6 ± 5.9%	5	58.4 ± 3.87 168.8 ± 11.2%*	5	8.8 ± 1.2*
3rd	5	7.08 ± 0.76 71.5 ± 7.6%	4	67.75 ± 7.08 195.8 ± 20.4%*	5	14.5 ± 0.5
5th	5	9.45 ± 0.7 95.4 ± 7.1%	4	36.0 ± 6.72 104.0 ± 19.4%	4	13.9 ± 1.0
7th	5	14.71 ± 1.12 148.6 ± 1.1%	4	69.38 ± 13.68 200.5 ± 39.5%	5	16.8 ± 0.7
9th	5	7.98 ± 1.02 80.6 ± 10.3%	4	49.5 ± 6.38 143.1 ± 18.4%	5	15.8 ± 0.6
14 days hypokinesia						
Control	12	9.90 ± 0.45 100.0 ± 4.5%	8	34.6 ± 3.3 100.0 ± 9.5%	15	15.9 ± 0.6
1st day	4	7.3 ± 0.58 73.7 ± 5.8%*	4	69.85 ± 4.06 201.9 ± 11.7%	5	9.0 ± 0.8
5th	4	5.5 ± 0.77 55.6 ± 7.7%*	4	61.35 ± 5.01 177.3 ± 14.4%	5	8.5 ± 1.6
7th	4	5.6 ± 0.75 56.6 ± 7.6%	3	54.4 ± 14.4% 156.0 ± 2.9%	4	11.9 ± 0.8
9th	4	4.89 ± 3.25 49.4 ± 3.2%*	4	88.45 ± 6.23 255.6 ± 18%*	4	9.6 ± 1.4

* Significant difference at P<0.05

Table 3

Dynamic of changes in the level of cyclic nucleotides in the blood plasma and their excretion in urine of 12 - month old rats as a result of 7 and 14 days hypokinesia.

7 days hypokinesia Hypokinesia Duration	Plasma nmol/ml				Urine nmol/100 g. day			
	No.	cAMP	No	cGMP	No.	cAMP	No.	GMP
Control	12	18.8 ± 1.3	12	8.2 ± 2.7	23	24.7 ± 1.5	23	9.7 ± 2.4
1st day	5	21.4 ± 1.2****	5	6.2 ± 0.3**	5	74.6 ± 1.5*	5	6.6 ± 2.8****
3rd	5	50.7 ± 1.4*	5	6.5 ± 0.2****	5	74.5 ± 1.3*	5	3.2 ± 0.2***
5th	5	16.2 ± 1.0***	5	2.6 ± 0.2**	5	63.7 ± 1.4**	5	1.6 ± 0.2**
7th			5	5.2 ± 0.2***	5	58.3 ± 1.2**	5	3.1 ± 0.3***
9th	5	12.7 ± 1.0	5	5.8 ± 0.2***	5	35.5 ± 1.3***	5	4.8 ± 1.0**
14 days hypokinesia								
Control	5	12.5 ± 1.6	24	9.5 ± 0.9	23	24.5 ± 1.0	2	7.0 ± 1.1
1st day	5	19.1 ± 1.3**	5	6.5 ± 0.8***	5	52.8 ± 2.0**	3	6.6 ± 1.0****
3rd	5	48.0 ± 1.3	5	8.1 ± 1.0****	5	77.6 ± 1.8*	5	1.8 ± 0.1***
5th	5	23.9 ± 1.3**	5	3.1 ± 0.7***	5	71.6 ± 1.1*	5	0.9 ± 0.1***
7th	4	7.5 ± 1.2**	8	3.4 ± 0.9**	6	64.3 ± 2.0**	6	2.3 ± 0.2***
9th	4	13.7 ± 1.2****	4	4.1 ± 1.0*	4	37.5 ± 1.3***	4	0.3 ± 0.1**

* $p<0.01$; ** $p<0.02$; *** $p<0.05$; **** $p>0.05$

Table 2 shows that the concentration of calcium in the blood plasma significantly changed only during re-adaptation period in the 1-year-old animals. Decreased calcium levels were registered in all stages in which a maximum decrease was observed at 5th and 9th days during 14 days of hypokinesia while after 7 days effect, a significant decrease of plasma calcium levels was observed only on the first day; the concentration was recovered, although this process occurred much slower in younger animals.

DISCUSSION

The results in Table 1 show that functional adaptation of nephrons to calcium and phosphate occurred more or less in the opposite phases after equal intervals of hypokinesia. At this time re-absorption system for calcium was less adaptable than similar system for phosphate. The results from the younger animals, showed that phosphate excretion was generally lower than in one year old animals (8.4 ± 0.5 µmol/day) This shows that the tendency to enhance phosphaturia was marked only on the 5th day of the adaptation, while the levels of the parameter normalised towards the 9th day of the condition. The young animals excreted less calcium (0.95 ± 0.2 µg/day) than the one year old ones. However its intensive excretion with urine towards the end of the re-adaptation period not only stopped but also increased up to 3.9 ± 0.5 µg/day.

The findings in Table 1 and 2 suggest that changes in calcimea, calciuria and phosphaturia in all animals were determined by the re-absorption process within the bone tissue during re-adaptation period of hypokinesia. This is supported by the following facts: enhanced excretion of UAC with urine (28.5 ± 1.1 µg/L day on the first day during 14 days hypokinesia as compared with 7.1 ± 0.6 µg/L day in the controls), elevated levels of blood sialic acid (16.51 ± 0.35 and 15.72 ± 1.54 mmol/L on the 9th day of the 14 and 7 days hypokinesia respectively as compared with 14.06 ± 0.42mmol/L in controls), and also as a supprise, a decrease in bone tissue (up to 0.094 ± 0.004 g/100 mg in the pelvic bone and up to 0.132±0.004 g/100 mg vertebrate tissue as compared with 0.142 ± 0.009 g/100 mg in the controls of the waist bone and 0.249 ± 0.010 g/100 mg in control of the vertebrate tissue) in the body of 12 month old rats. A similar phenomenon was observed in young rats, i.e. enhanced excretion of UAC with urine (13.2± 1.6 µg/L day after the first day during the 7 days of hypokinesia. The latter was compared with 9.6 ± 1.3 µgL day for the same age group of the controls. However, normalisation of these parameters occurred faster in these younger animals than in the older ones.

Analysis of the dynamic of changes in the ratio of cAMP/cGMP shows that a maximum value of this relationship was observed between the 3rd and 5th day of hypokinesia in 12 month old animals. Therefore, the re-adaptation that was caused by hypokinesia was accompanied by a transition from relatively low intensive action of adrenergic agents on metabolic process in the organism to their enhanced action (or to the increased response of the signals in the receptor systems). At the same time (judging by the dynamic of changes in cAMP) cholinergenic impulses were weakened. The value of the urine cAMP/cGMP for one-year-old rats, which were considered after 14 days of hypokinesia, was significantly high after the 9th day compared with the same age group. This significant change towards dominance of adrenergic phase of regulation is probably associated with the fact that the repeated stimulation phase of metabolic regulation occurred at a latter stage of post hypokinetic re-adaptation of kidney tissues. Judging by the plasma PTH levels and the amount of excreted calcium under these conditions, PTH practically stopped playing the role of regulating the activity of nephrotic cells. This may be an evidence of decreased intensity of tubular re-absorption for calcium and a delayed release of phosphate. When comparing the changes in the concentration of hormones, cyclic nucleotides and calcium, we can conclude that under the described conditions, CT (probably) plays the role of a modulator of cAMP metabolism. This is not in line with the well-known suggestion(14, 20) about the relationships between calcitonin and the system of cAMP metabolism. In contrast, PTH works together with the system of cGMP metabolism(21, 22).

Analysis of a large number results that were obtained while conducting experiments with young rats, shows that animals of this age group are normally characterised by a specific individuality of their biochemical status. However, this unique feature was significantly observed when stress factors acted on the organism. This phenomenon is clearly depicted by studying dynamics of excretion of nucleotides with urine and by the variation of their concentration in blood. Thus, it was established that the excretion of cAMP with urine by three months old rats (although only on the first day of re-adaptation period) was 88.1 ± 2.3mnol/100 g day (after 7 days of hypokinesia). At that time a maximum value of this parameter in one year-old rats was 74.6 ± 1.5 nmol/100 g day under the same conditions (Table 2). Towards the 9th day, this value was 31.0 ± 1.8 nmol in young rats against 27.1 ± 0.9 nmol/100 g day in controls, in 12 month old rats 35.5 ± 1.3 nmol/100 day against 24.7 ± 1.5 nmol/100 day in controls.

Noteworthy, the correlation between the amount of phosphate and creatinine which are secreted with urine. The registered variation may be due to characteristic changes of nitrogen metabolism in the skeletal muscle(21-23). This was observed in animals of all age groups, almost at every interval during the course of 14 days of hypokinesia, except after the 7th day, when the values of these parameters changed in the opposite phases). The reported variations may also be due to the characteristic changes in nitrogen metabolism within the skeletal muscle. Therefore, it seems that during the pathogenesis of hypokinesia, the most critical moment is its initial stage when the functions of the cells at the levels of reception and transmission of information are balanced. Our results show that, the blood composition of PTH and their messengers (calcium and nucleotides) change during hypokinetic period, probably due to feedback inhibition mechanism of their synthesis in the respective endocrine organs.

CONCLUSION

Analysis of these results shows that a great change in metabolism of bone tissue and mineral metabolism in the whole organism occurs before the "exit" of hypokinesia. An adaptation phase can be depicted judging from the fact that from this state of negative mineral balance, the levels of re-absorption stimulator (PTH) and PTH antagonist were significantly reduced and increased respectively. Thus, we suggest that in future such studies be conducted just at the earlier stages of hypokinesia. Besides, the comparative results obtained from young animals (3 month old) and old animals (12 month old) enable us to think that the body of older animals is characterised by slightly higher ability of hypokinetic conditions, when a wide range of metabolic changes are observed in younger animals.

ACKNOWLEDGEMENTS

I wish to thank my laboratory assistant for having participated tirelessly during the experiments. I am also grateful to Prof A.N Kaliman, the Chairman of Biochemistry department (Kharkov State University) for his encouragement.

REFERENCES

1. Kovalenko, E.A. and Gurovsky N.N. Hypokinesia. *M Medicina.* 1980; 319p.
2. Larina I.M., Popova I.A, Mikhailov V.M. and Bulavkova. The hormonal mechanisms supporting muscle work during prolonged head-down tile hypokinesia. *Fiziol Cheloveka.* 1999; **25**:117-124.
3. Gushchin V.I., Pustynnikov I.V.M., Efimov V.A., Smirnova T.M., and Koshelev V.V. Dynamics of interrelationship in a small group during long term hypokinesia. *Aviakom Ekolog Med.* 2000; **34**:24-29.
4. Khachaturian, Z.S. The role of calcium in brain aging. Re-examination of hypothesis. *Aging.* 1989; **1**:17-34.
5. Gibson, G.E and Peterson C. Calcium and aging nervous system. *Neurobiology Aging* 1987; **8**: 324-344.
6. Landfield, P.W. "Increased calcium current" hypothesis of brain aging. *Neurobiology. Aging.* 1987; **8**:346.
7. Landfield, P.W., Campbell, L.W., Hao, S.Y. and Kerr, D.S. Aging related to mechanisms of brain aging and Alzheimer's disease. In: Calcium, membranes, Aging, and Alzheimer's disease. Z.S Khachaturian, C.W. Cotman and J.W Pettergrew. Eds. *Ann. NY Acad. Sc.* 1989; **568:** 95-105.
8. Landfield. P.W., Thibault, O, Mazzanti, M.L., Porter, N.M. and Kerr, D.S. Mechanism of neural death in brain aging and Alzheimer's disease: Role of endocrine-mediated calcium dyshomeostasis. *J. Neurobiol.* 1992; **9**:1247-1260.
9. Verkhratsky, A., Shimigol, A., Kirichuk, S., Prochuk, N. and Kostyuk, P. In: Calcium hypothesis in Aging and Dementia. Eds. Disterhorft, J.F, Gispen W.M., Traber, T, Khachaturian Z.S. *Ann. NY Acad. Sc.* 1994; **747**:365-381
10. Fedulova, S.A., Kostyuk P.G. and Veselovsky N.S. Ionic mechanism of electrical excitability in rat sensory neurons during postnatal ontogenesis. *Neuroscience.* 1991; **41**: 303-309.
11. Franklin J.L. and Johnson E.M. Jr. In: Calcium hypothesis for Aging and Dementia. Eds. Disterhorft J.F., Gispen W.M., Traber T., Khachaturian Z.S. *Ann. NY Acad. Sc.* 1994; **747**: 195-204.
12. Perceva M.N., Sheludkova Z.P, and Kuznetsova L.A. The trends of development in hormone-reactive enzymatic systems of metabolism and functions of cyclic adenosine monophosphate. In: Cyclic nucleotides. Moscow. Nauka: 1979; 46-54.
13. Dorofeev G.I, Koshemiakin L.A. and Ivashkin V.T In: Cyclic nucleotides and adaptation of organism. L. Nauka. 1980; 220p.
14. Fedorov N.A. Biological and clinical significance of cyclic nucleotides. *M. Medicina.* 1979; 200p.
15. Steiner A.L., Pagliari A.S., Chase L.R., and Kipnis D.M. Radioimmunoassay for cyclic nucleotides. II. Adenosine-3', 5'monophosphate in mammalian tissues and body fluids. *J. Biol. Chem.* 1972; **234**:1114-1120.
16. Doctorovich N.L. Determination of calcium in bone tissue using trilon. In: Physiology and Biochemistry of animals. 1972; **5**:114-117
17. Taussky H.H. and Shorr E. A microcalorimetric method for the determination of inorganic phosphorus. *J. Biol. Chem.* 1953; **202**:675-685.
18. Bosnes R.W. and Tussky N.N. On calorimetric determination of creatinine by the Jaffe reaction. *J. Biol. Chem.* 1945; **158**:581-591.
19. Bitter T. and Muir. A modified uronic acid carbasone reaction. *Anal. Biochem.* 1962; **4**:330-334.
20. Kolb V.G. and Kamishnikov V.C. Test on Sialic acids according to the method of Gess. In: Clinical Biochemistry. Minsk. Belorussia 1976; 145-147.
21. Bulatov A.A. Parathyroid hormone and calcitonin. In: Biochemistry of hormones and hormonal regulation. M. Nauka. 1976; 378p.
22. Zukov E.K., Barabashova Z.S. and Fedorov V.R. The effect of hypokinesia on functional state of skeletal muscle. *J. Physiol. USSR.* 1971; **9**:1240-1245.
23. Lehninger A.L., Nelson D.L and Cox. Principals of Biochemistry. 2nd Ed. CBS Publishers and Distributors. 1993.