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EFFECTS OF COBALT IONS ON THE SYNOVIAL PRODUCTION OF NEUTRAL PROTEINASES AND PROSTAGLANDIN E₂

INTRODUCTION

Artificial joint replacements contain much metal. For a number of years the metal of choice has been an alloy based on cobalt, although newer prostheses are being made from titanium. The major cause of failure of these devices is aseptic loosening. Recent evidence suggests that loosening is secondary to a localized loss of bone in the area which surrounds the implant. Examination of the femoral component of failed artificial hip joints reveals the presence of a membrane growing at the surface where the surrounding bone meets the cement used to secure the prosthesis [1]. This membrane bears striking histological and cellular similarities to the synovial membrane which lines all articulating joints. Furthermore, it secretes collagenase and prostaglandin E₂ (PGE₂) in culture, thus implicating this pseudo-synovial membrane in the localized osteolysis that promotes aseptic loosening. If so, it is important to identify the local stimuli that provoke the production of collagenase and PGE₂ by cells of the synovial type. Working on the hypothesis that metal ions released by the prosthesis may be responsible, we are engaged in screening various implant metals for their ability to stimulate the production of PGE₂ and three neutral proteinases, including collagenase, by synovial cell cultures. Here we report the effects of Co²⁺.

METHODS

Synovia were obtained from human or lapine knee joints and cultured by standard methods [2]. Sterile, aqueous solutions of CoCl₂ were added to confluent cultures to give final metal ion concentrations from 0 to 10⁻³ M. After 3 days further incubation, the conditioned media were assayed for their neutral proteolytic activity, using ³H-collagen, -gelatin or -casein as substrates and aminophenylmercuric acetate (1 mM) as activator. Prostaglandin E₂ levels were measured by radioimmunoassay.

RESULTS AND DISCUSSION

The production of all three neutral proteolytic activities was stimulated by Co²⁺. For lapine cells, the maximum stimulation of 10-30 fold occurred in the presence of 10⁻⁷ M Co²⁺ (Table 1). Human cells required 10⁻⁴ to 10⁻⁵ M Co²⁺ to achieve a maximum stimulation of 10-15 fold. Production of PGE₂ by lapine cells was elevated 1.5-2 fold at concentrations of Co²⁺ that maximally provoked enzyme release, whereas all concentrations of Co²⁺ slightly depressed the synthesis of PGE₂ by human synovial cells.

Table 1

Production of neutral proteinases and PGE₂ by lapine synovial cells in response to Co²⁺

Metal Ion Added	Enzymic Activity (Units/day/10 ⁶ cells*)			
	Collagenase	Gelatinase	Caseinase	PGE ₂ (ng/culture)
None	0.40	0.27	0.050	193
Co ²⁺ (10 ⁻⁷ M)	13.23	3.96	0.613	285

* 1 Unit of neutral proteinase activity degrades 1μg of the appropriate substrate per min at 37°C.

Suitable control experiments confirmed that Co²⁺ mediated its apparent effects on the production of these neutral proteinases by stimulating the cellular synthetic machinery. Concentrations of Co²⁺ that maximally enhanced apparent enzyme production had no effect when added directly to the proteinase assays. In addition, dialysis of control conditioned media against various concentrations of Co²⁺ failed to stimulate the enzymes' activity post-synthetically. Furthermore, cells whose pro-

duction of neutral proteinases had been previously stimulated by the ionophore A23187 failed to become «hyperstimulated» in the presence of Co^{2+} (data not shown).

As the concentrations of Co^{2+} needed to produce these effects are found in patients with prosthetic joints [3,4], the cellular reactions we describe here deserve further scrutiny.

REFERENCES

- [1] S.R. GOLDRING, A.L. SCHILLER, M. ROELKE, D.A. O'NEILL, W.H. HARRIS, *J. Bone Joint Surg.*, **65A**, 575-584 (1983).
- [2] C.H. EVANS, J.D. RIDELLA, *Anal. Biochem.*, **142**, 411-420 (1984).
- [3] R.F. COLEMAN, J. HERRINGTON, J.T. SCALES, *Br. Med. J.*, **1**, 527-529 (1973).
- [4] H.S. HOBBS, M.J. MINSKI, *Biomaterials*, **1**, 193-198 (1980).



PS6.20 — MO

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THE STRUCTURE AND REACTIVITY OF Ni(II)-ALBUMIN PROTEIN COMPLEX

There is much concern regarding the toxic effects of ingested nickel, particularly among workers in nickel refineries [1,2]. For this reason the biochemical studies undertaken by SARKAR and his co-workers [3,4] in relation to ingested Ni are of particular importance. They have shown that the main Ni(II)-binding constituents in human blood are the amino acid histidine (His) and the protein albumin. This distribution resembles that for Cu(II) except the albumin has a much stronger affinity [4] for Cu(II) than Ni(II). Nevertheless,

these studies have established that the two metal ions bind to the same site in the albumin, and this, from Cu(II) studies, is known to involve the *N*-terminal amino acid residues. The proposed near square-planar geometry formed from the amino *N*, deprotonated peptide *N* atoms, and a histidine *N* is depicted in Fig. 1.

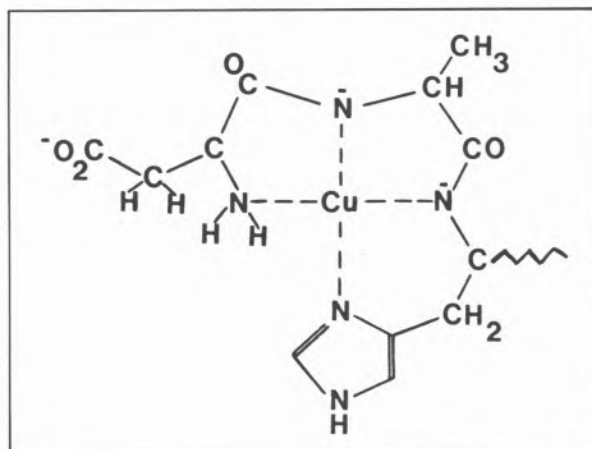


Fig. 1

The proposed Cu(II) coordination site

The ability of Ni(II) to cause peptide *N* deprotonation is known to be less than that of Cu(II), and a structure such as that in Fig. 1 would require a high pH for its formation. In keeping with this the UV/Vis absorption spectrum of 1:1 Ni(II):albumin shows an intense absorption band at 420 nm, characteristic of a square-planar environment, which reaches a maximum absorption at $\text{pH} > 9$ [4].

We have examined the bovine albumin binding of Ni(II) by means of UV/Vis absorption and CD spectroscopy. In agreement with GLENNON and SARKAR [4] the square-planar type spectra reach a maximum at $\text{pH} > 9$. The spectra, shown in Fig. 2, reveal that at the pH of blood (7.4) 70% of the Ni is bound as in Fig. 1. The remaining 30% must be octahedrally co-ordinated since, under the conditions used, this would be spectroscopically silent. The rapidity of interconversion between the two forms as the pH is altered suggests that the octahedral site must also be at the *N*-terminal end of the protein chain.

We have also found that the rate of ligand exchange for Ni(albumin), as in

