ADVANCES IN PHARMACOLOGY AND THERAPEUTICS

Proceedings of the 7th International Congress of Pharmacology

General Editors: J. R. BOISSIER, P. LECHAT & J. FICHELLE

Volume 3

IONS-CYCLIC NUCLEOTIDES-CHOLINERGY

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Editor

J.C. STOCLET
Strasbourg

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Introduction

The scientific contributions at the 7th International Congress of Pharmacology were of considerable merit. Apart from the sessions organised in advance, more than 2,200 papers were presented, either verbally or in the form of posters, and the abundance of the latter in the congress hall is a good indication that this particular medium of communication is becoming increasingly attractive to research workers, and offers scope for discussions which combine an elaborate, thorough approach with a certain informality.

It would have been preferable to have published the entire congress proceedings within the framework of the reports. That was, however, physically impossible, and the organisers had to adopt a realistic solution by publishing only the main lectures, symposia and methodological seminars. The amount of material presented necessitated the printing of ten volumes, each volume containing congress topics regrouped according to their relevant content and subject areas. This system of division may give rise to criticism on account of its artificiality, and we readily admit that certain texts could have been placed in more than one volume. We are asking the reader to excuse this arbitrariness, which is due to the editors' personal points of view.

I draw attention to the fact that most of the symposia finish with a commentary which the chairmen had the option of including, presenting their personal opinions on one or several points. We think that such an addition will facilitate reflection, discussion, indeed even controversy.

The launching of the scientific programme for this congress began in September 1975 on returning from the last meeting in Helsinki. Long and delicate discussions took place in the Scientific Programme Committee and with the International Advisory Board. Should it be a pioneer, 'avant-garde' congress? Or one laid out like a balance-sheet? Should we restrict the congress to the traditional bounds of pharmacology, or extend the range of papers to cover the finest discipline? The choice was difficult, and the result has been a blend of the two, which each participant will have appreciated in terms of his training, his tastes, and his own research.

A certain number of options, however, were taken deliberately: wide scope was given to toxicology, from different points of view, and to clinical pharmacology, a subject much discussed yet so badly practised; the founding of two symposia devoted

to chemotherapy of parasitic diseases which are still plagues and scourges in certain parts of the world; a modest but firm overture in the field of immunopharmacology, which up until now was something of a poor relation reserved only for clinical physicians; the extension of methodological seminars, in view of the fact that new techniques are indispensable to the development of a discipline.

We have been aware since the beginning that, out of over 4,000 participants who made the journey to Paris, not one could assimilate such a huge body of knowledge. Our wish is that the reading of these reports will allow all of them to become aware of the fantastic evolution of pharmacology in the course of these latter years. If one considers pharmacology as the study of the interactions between a "substance" and a living organism, then there is no other interpretation. Nevertheless, one must admit that there exists a period for describing and analysing a pharmacological effect, and that it is only afterwards that the working mechanism can be specified; a mechanism which will permit these "substances" to be used for the dismantling and breaking down of physiological mechanisms, a process which justifies Claude BERNARD'S term, "chemical scalpel".

The reader will be able to profit equally from more down-to-earth contributions, more applied to therapeutics, and less "noble", perhaps, for the research worker. He will realise then that his work, his research and his creative genius are first and foremost in the service of Man, and will remember this statement from Louis PASTEUR:

> "Let us not share the opinion of these narrow minds who scorn everything in science which does not have an immediate application, but let us not neglect the practical consequences of discovery."

I would like to renew my thanks to my colleagues in the Scientific Programme Committee and also to the members of the International Advisory Board, whose advice has been invaluable. I owe a particular thought to J J BURNS, now the past-president of IUPHAR, who granted me a support which is never discussed, and a staunch, sincere friendship. The Chairmen have effected an admirable achievement in the organisation of their proceedings, and in making a difficult choice from the most qualified speakers. The latter equally deserve our gratitude for having presented papers of such high quality, and for having submitted their manuscripts in good time.

The publisher, Robert MAXWELL, has, as always, put his kindness and efficiency at our service in order to carry out the publication of these reports. But none of it would have been possible without the work and competence of Miss IVIMY, whom I would like to thank personally.

My thanks again to the editors of the volumes who, in the middle of the holiday period, did not hesitate to work on the manuscripts in order to keep to the completion date.

Finally, a big thank you to all my collaborators, research workers, technicians and secretaries who have put their whole hearts into the service of pharmacology. They have contributed to the realisation of our hopes for this 7th International Congress, the great festival of Pharmacology. Make an appointment for the next one, in 1981, in Tokyo.

Jacques R BOISSIER

Chairman

Scientific Programme Committee

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The Control of Calcium Metabolism in Health and Disease

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SUMMARY

In man the body's calcium economy is determined by the relationship between the intestinal absorption of calcium, renal handling of calcium and the movements of calcium in and out of the skeleton. The regulation of calcium metabolism involves three distinct but inter-related aspects: 1) The control of the concentration of calcium in extracellular fluid and tissues; 2) The control of the body's overall calcium balance, i.e., the relationship between gains and losses; and 3) The control of the shape, structure and composition of bone and the way these respond to external factors such as load bearing. These processes are influenced by many factors. The principal calcium regulating hormones are parathyroid hormone, the renal metabolites of vitamin D (notably 1,25-dihydroxy vitamin D3), and calcitonin, although the role of the latter in man is still controversial. The secretion of these major regulating hormones is determined mainly by calcium and phosphate levels. Many other hormones have some influence on calcium metabolism; these include thyroid hormone, prolactin, growth hormone, insulin, the somatomedins, and the adrenal and gonadal steroids. The major clinical disorders of calcium metabolism either involve disturbances in the secretion or action of these hormones, or are due to disturbances in bone metabolism itself. Several drugs have some effect on calcium metabolism but much remains to be learnt about the pharmacology of the skeleton. Perhaps the greatest therapeutic challenge is presented by osteoporosis, which is a major cause of disability and fractures in the elderly.

INTRODUCTION

In recent years there has been a rapid advance in knowledge about calcium metabolism. In particular, the chemical nature of the major calcium regulating hormones has been determined and their mode of action clarified. This recent work has led to a re-examination of many older concepts and the new knowledge has yet to be fully applied to human disease.

DISTRIBUTION OF CALCIUM AND PHOSPHATE

The total body content of calcium is between 1-1.5 kg and for phosphorus 0.7-1 kg

for an average adult weighing 70 kg. The skeleton contains about 98% of this calcium and 85% of the phosphorus. Studies with radioisotopes (^{45}Ca and ^{47}Ca) indicate that in normal human adults the exchangeable pool of calcium represents less than 1% of total body calcium. This exchangeable pool of calcium is very important in homeostasis and about half of it lies outside the skeleton. Continuous movement occurs of calcium ions between body fluids, cells and the surface of bone. Skeletal renewal occurs at a rate approximately 1-4% of the total adult skeleton each year. Trabecular bone has a faster turnover than cortical bone.

Extracellular Fluid Calcium

The total concentration of calcium in plasma is normally between 2 to 2.5 mmol/litre. Of this about 1.2 mmol/litre is present as ionised calcium (Ca^{2+}), the remainder is bound to proteins, particularly albumin, and to small ions such as citrate and phosphate. It is the ionic Ca^{2+} which is regulated. Under most conditions Ca^{2+} bears a constant relationship to total plasma calcium. The concentration of extracellular Ca^{2+} is important for neuromuscular function and many other biological events, e.g., blood coagulation.

Intracellular Calcium

In most, if not all, cells, cytosol concentrations of Ca^{2+} are maintained at much lower concentrations than extracellular and are in the range of 10^{-5} to 10^{-6} mols/litre. Within cells, mitochondria are capable of accumulating large amounts of calcium against electrochemical gradients to the point at which intramitochondrial deposits of insoluble calcium phosphate can form. Intracellular calcium concentrations are of critical metabolic importance and changes in intracellular calcium frequently accompany changes in cell function, e.g., in response to hormones. There is a complex inter-relationship between intracellular Ca^{2+}, 3'5'-cyclic AMP and cell activation in response to many external stimuli.

Major Fluxes of Calcium and Net Calcium Balance

Three major organs are involved in the regulation of calcium metabolism. These are the gut, kidney and bone. These three organs can account for the major gains and losses of calcium from the external environment and for the exchange of calcium in and out of the extracellular fluid.

The only significant route of entry of calcium into the body is by intestinal absorption. The true absorption of calcium is greater than the net absorption because some calcium is returned to the intestinal lumen in biliary, pancreatic and intestinal secretions. The body loses calcium by urinary excretion and in sweat. The latter is usually ignored in balance studies because the losses are usually small and difficult to measure.

Inspection of the size of the fluxes shows that the kidney handles the largest amounts of calcium per day in terms of filtered load and tubular reabsorption.

In the adult under normal conditions, the body is neither gaining nor losing calcium, so that inflow and outflow are matched precisely (intake = output). In disease states there may be transient or sustained net gains or losses, to produce calcium balances that are positive (intake exceeds output) or negative (output exceeds intake).

During growth there is a net daily gain to provide the calcium necessary for skeletal growth. During pregnancy or lactation, the fetus or child gains calcium from the mother. Under these various conditions, the extra requirements for calcium are met by increased net intestinal absorption and diminished renal excretion, so that a neutral balance can be maintained.

There are two important aspects of calcium homeostasis. The first is concerned with the body's overall balance which is determined by the relative rates of flux through these organs and the complex way in which they are changed by regulating factors, particularly parathyroid hormone (PTH), calcitonin (CT) and vitamin D (Vit D). The second aspect is concerned with the control of the concentration of calcium and phosphate (Pi) in extracellular fluid (ECF); this control can often be independent of the total body economy. The control of ECF concentrations is achieved by changes in the relative sizes of the various fluxes and by the level of Ca^{2+} and P_i at which the various controlling agents are switched on and off

CALCIUM REGULATING HORMONES

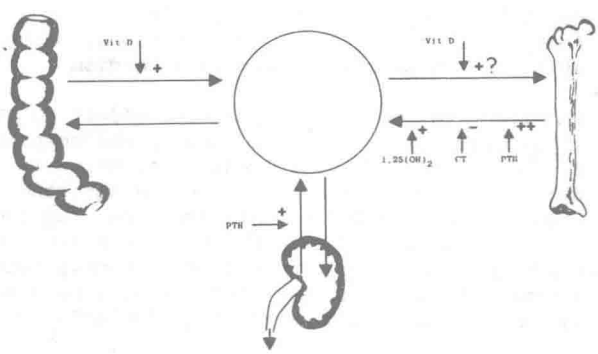

Fig. 1. The principal movements (fluxes) of calcium in an adult human and the major sites of hormonal control

HORMONES ACTING ON CALCIUM METABOLISM

The hormones can be divided into "controlling" hormones and "influencing" hormones. The controllers are the primary calcium regulating hormones, PTH, CT and Vit D metabolites, the secretion of each of which is altered in response to changes in plasma ionised Ca concentration (and phosphate in the case of Vit D). They have their major effects on plasma Ca and phosphate by altering the fluxes of these ions between the ECF pool and gut, kidney and bone. The "influencing" hormones are those other hormones, e.g., thyroid hormones, prolactin, growth hormone, insulin, somatomedins, and adrenal and gonadal steroids, which have effects on calcium metabolism but whose secretion is determined primarily by factors other than changes in plasma calcium and phosphate. These hormones tend to have their major effects on the skeleton itself rather than on the regulation of plasma Ca.

THE MAJOR CONTROLLING HORMONES

Parathyroid Hormone (PTH)

Mammalian PTH consists of a single peptide chain, 84 amino acids long, for which the entire sequence is now known for several mammalian species, including man. It circulates in plasma mainly as fragments of this molecule. Biological activity resides in the first 32 to 34 amino acids reading from the N-terminal end. The major stimulus to its secretion is a fall in plasma Ca^{2+}, whereas a rise in plasma Ca^{2+} suppresses PTH secretion. Other ions, e.g., Mg^{2+}, only play a minor role in regulating secretion.

The major action of PTH is on the kidney, to increase renal tubular reabsorption of calcium and to depress the tubular reabsorption of phosphate. This leads to a rise in plasma Ca and a fall in plasma P_i. The detailed biochemical basis for these actions is not clear, although the effect on phosphate secretion seems to involve activation of an adenylate cyclase to produce cyclic AMP within the renal tubule. This renal receptor mechanism appears to be defective in pseudohypoparathyroidism, since patients with this inherited disorder show no renal response to PTH in terms of increased excretion of phosphate or cyclic AMP.

PTH also diminishes the secretion of H^+ by the kidney, which leads to the excretion of HCO_3 ions and to a hyperchloraemic acidosis, which is often present to some degree in patients with primary hyperparathyroidism.

It is extremely important to note that in man the effect of PTH on plasma calcium is mediated mainly by increasing the renal tubular reabsorption of calcium. The better known effect of PTH on bone to increase resorption is relatively less important in producing changes in plasma calcium because the net fluxes of Ca in and out of the skeleton are relatively small compared with the large fluxes across the kidney. The bone resorbing effect of PTH is best seen only under pathological conditions, e.g., in primary and secondary hyperparathyroidism. Nevertheless, there is some evidence that low doses of PTH, possibly within the physiological range, may have an important effect in stimulating bone formation and bone turnover.

The effect of PTH to increase intestinal absorption of calcium is now thought to be an indirect action brought about by the ability of PTH to increase the renal synthesis of 1,25-dihydroxycholecalciferol.

Calcitonin (CT)

CT is a peptide hormone, which contains 32 amino acids with a cysteine-dependent disulphide bridge between positions 1 and 7. Although there are striking differences between the amino acid composition of CTs from different species, CT from one species commonly exerts biological effects in others. CT is secreted by specialised cells, designated C-cells, which are part of the APUD cell series derived embryologically from the neural crest. In man, C-cells reside mainly in the thyroid.

CT is secreted in response to a raised plasma Ca^{2+} but other factors also stimulate its secretion, e.g., gastrointestinal peptide hormones, alcohol and β-adrenergic agents. Like PTH it is heterogenous in the peripheral circulation.

The most important action of CT in mammals is to inhibit bone resorption and thereby lower plasma calcium and phosphate. Large doses of CT also increase renal excretion of calcium, and gut secretion of calcium, sodium and phosphate

and alter the soft tissue distribution of these ions, but these are probably not significant effects under physiological conditions. There is some evidence that in other species CT may have other roles, for example, in regulating the availability of Ca from bone during egg laying in birds and in controlling Ca and sodium homeostasis in migratory fish.

The exact physiological role of CT in man is unclear. Some, but not all, radioimmunoassays can detect circulating CT in normal man and can show enhanced secretion in response to hypercalcaemia, or other provoking agents, e.g., alcohol. Recent evidence suggests that relative deficiency of CT may contribute to the bone disease seen in chronic renal failure, and possibly to other diseases. CT is secreted in excess by medullary carcinomas of the thyroid and can be used for the detection of pre-symptomatic disease in family members. There is also evidence that CT is secreted in excess in the various other tumours, e.g., bronchial carcinomas.

CT is used in man to reduce the excessive resorption and turnover of bone characteristic of Paget's disease and also to treat hypercalcaemia of malignancy.

Vitamin D (Vit D)

Animals derive their Vit D from the diet and from ultraviolet irradiation of 7-dehydrocholesterol in the skin. The essential steps in its subsequent metabolism are conversion to 25-hydroxycholecalciferol in the liver and the subsequent production of various dihydroxy metabolites in the kidney and elsewhere. The most important metabolite is probably $1,25(OH)_2D_3$, which has the major actions of the parent vitamin on intestine, bone and muscle. The intestinal absorption of Ca appears to involve both active transport and diffusion processes. Of the Ca regulating hormones, $1,25(OH)_2D_3$ is the most important in influencing intestinal absorption of calcium and phosphate. The precise mechanism of stimulation is unclear but seems to involve the synthesis of a calcium binding protein as well as a calcium-stimulated ATPase on the brush border surface of intestinal epithelial cells. The adjustment of Ca and phosphate absorption to body requirements and to changes in dietary intake seems to involve appropriate changes in the production of $1,25(OH)_2D_3$. One important feature of the control of Ca and phosphate absorption by $1,25(OH)_2D_3$ is that the adaption is relatively slow and takes several hours or days to come about, which is quite unlike the rapid responses of the kidney and bone to PTH and CT.

Intestinal absorption of Ca varies with age and sex and in various disease states, and some of these changes may reflect differences in either the production of $1,25(OH)_2D_3$, or in the target tissue responses to it. Although lack of vitamin D in man is associated with defective mineralisation of cartilage and bone, the question of whether Vit D or its metabolites act directly on skeletal tissues to increase mineralisation remains unsettled. It is possible that the effects of Vit D on bone mineralisation are secondary to changes in extracellular fluid concentrations of calcium and phosphate. The major effect of $1,25(OH)_2D_3$ on bone in culture is to increase resorption and this may contribute to making Ca and phosphate available for mineralisation. This effect of $1,25(OH)_2D_3$ on bone resorption probably occurs with physiological doses and $1,25(OH)_2D_3$ may be an important natural regulator of bone resorption.

Muscle weakness is also a well recognised feature of Vit D deficiency in man. It is possible that Vit D, or its metabolites, have direct effects on muscle function but little is known about this action.

The production of $1,25(OH)_2D_3$ by the kidney is closely regulated by a number of

factors, including Vit D status, dietary calcium and phosphate, PTH, prolactin, growth hormone and oestrogens.

The factors which control Vit D metabolism in man require further elucidation but there is some evidence that the factors referred to above have some influence. Thus $1,25(OH)_2D_3$ levels are high in patients with hyperparathyroidism and low in hypoparathyroidism, indicating the possible influence of PTH or phosphate. Similarly, the low levels in chronic renal failure may indicate suppression by high plasma phosphate, or could be due to diminished renal mass. When the body's demand or requirements for Ca or P_i are increased, the production of $1,25(OH)_2D_3$ appears to be enhanced. For example, the increased intestinal absorption of Ca in growth, pregnancy and lactation may be mediated by increased production of $1,25(OH)_2D_3$, brought about by prolactin, growth hormone or oestrogens.

THE ROLE OF THE SKELETON

The structural organisation of bone is extremely complex and it has proved a difficult tissue to study biochemically.

In mature bone, three main cell types exist: osteoblasts responsible for bone formation; osteoclasts responsible for bone destruction; and osteocytes which are derived from osteoblasts and become trapped within the bone matrix as maturation proceeds. The osteocytes lie within a complex canalicular system and are probably responsible for many of the rapid ion fluxes that occur in bone. The origin, life span and fate of various cells in bone is gradually being elucidated Osteoprogenitor cells may arise from marrow stroma, whereas osteoclasts are derived, in part at least, from wandering mononuclear cells.

Tissue fluid surrounding the bone cells probably has a unique composition and is high in K^+ and contains particular plasma proteins in preference to others.

The mineral component of bone is predominantly hydroxyapatite. Since ionic exchange occurs between bone mineral and surrounding fluids it also contains other ions such as HCO_3, Mg^{2+}, Na^+, K^+, etc. Collagen is a major constituent of the organic matrix of bone and is largely responsible for its tensile strength. In addition the matrix contains several glycosaminoglycan components. The rate of deposition of bone matrix is controlled by hormonal factors (eg, PTH, GH), or by levels of Ca and phosphate, and by mechanical and electrical forces acting on bone.

Calcification is an important step in the transition between matrix production and the formation of mineralised bone. Mechanisms of calcification may differ according to the site at which it occurs. In general, the concentrations of Ca and phosphate in ECF are too low to initiate deposition of calcium and phosphate but can sustain crystal growth once it has started. In cartilage and bone, the first steps in calcification are now thought to take place in or around small membrane-bound vesicles found in the matrix. These vesicles probably arise from the plasma membranes of hypertrophic chondrocytes during the maturation of epiphysial cartilage, or from osteoblast membranes in osteoid tissue. The vesicle membranes are rich in alkaline phosphatase, an enzyme which has been known for many years to be associated with calcification.

Rickets is a term used to define failure of mineralisation of cartilage in long bones and is seen in growing children. Osteomalacia is a histological appearance of unmineralised bone matrix and has many causes apart from Vit D deficiency. For example, phosphate deprivation, or hypophosphatemia due to renal tubular disorders (eg, Vit D resistant rickets), may be associated with a defect in skeletal mineralisation