JPP Journal of Pharmacy and Pharmacology

JPP 2002, 54: 1429–1431

Received February 21, 2002
Accepted June 20, 2002
ISSN 0022-3573

Retine revisited

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Abstract

Retine, so named by Albert Szent-Györgyi, an inhibitor of the growth of transplanted malignant tumours in animals, is present in all mammalian tissues and in urine. Its inhibitory activity was extensively investigated by Szent-Györgyi, but its exact chemical identity was not determined. Details of the reported physical and chemical properties of retine and its ubiquitous occurrence identify it as being identical to a complex mixture of lipid 2,4-diketones of similar ubiquitous occurrence. This lipid mixture has been extensively studied, and individual members have been synthesized.

In the 1960s, Albert Szent-Györgyi and his colleagues at the Institute for Muscle Research, Woods Hole, Massachusetts, USA were investigating a substance in certain extracts of mammalian tissues and of urine, which inhibited the growth of malignant tumours in experimental animals, an observation that he had been previously aware of (Szent-Györgyi 1967). He named this substance "retine" in distinction to another factor, "promine", which was also present, and which stimulated cell division and growth. Because of the ubiquitous glyoxalase system, which is present in all tissues, he made the assumption that retine might be a glyoxal derivative, the elusive substrate of this enzyme system, a substrate for which several eminent biochemists had searched in vain. His assumption was supported by the observation that infra-red spectra of purified tissue extracts had absorption bands of an aldehyde and a ketone, and possessed cancer inhibitory properties. Methylglyoxal was a known toxic metabolite, which was converted to lactic acid by the glyoxalase system. Since retine could be extracted from urine as well as from tissues, the former was a more convenient source of this substance (Hegyeli et al 1963). From its behaviour on Sephadex columns, its estimated molecular weight was in the region of 400 to 430. Its true chemical identity was never elucidated at that time, in spite of numerous attempts by Szent-Györgyi and others.

In 1965, Szent-Györgyi came to the conclusion that retine was indeed a glyoxal derivative, a substrate for the glyoxalase enzyme system. Based on his findings, he proposed a promine/retine theory of cell division, which included a role for the ubiquitous glyoxalase enzyme system (Szent-Györgyi 1965; Együd et al 1967). Szent Györgyi believed that retine might be a valuable agent in cancer research and in the treatment of cancer. A number of methylglyoxal analogues and other carbonyl-containing compounds were synthesized by his co-workers for testing for inhibition of tumour development.

In 1958, L. E. Francis and K. I. Melville, while investigating the aetiology of diphenylhydantoin-induced gingival hyperplasia in cases of epilepsy, employed the Barsoum & Gaddum (1935) guinea-pig ileum bioassay for histamine estimations in gingival tissue specimens. They noted the presence of a histamine-inhibiting substance in normal gingival tissue (Francis & Melville 1958). When I joined Drs Francis and Melville to assist in its chemical identification, we found that the principle in gingiva was identical to that present in all human and mammalian tissues (Douglas et al 1963). Consistent with the presence of the inhibitor demonstrated by bioassay was a single, well-defined area on paper chromatograms of tissue acid hydrolysates, which gave a strong positive reaction with 2,4-dinitrophenylhydrazine for C=O groups. Eluates of this region strongly inhibited histamine in the bioassay. From its chromatographic behaviour, infra-red and ultraviolet absorption spectra and other tests, we deduced that the inhibitor could possess a β-diketone structure. It was not possible to make further progress until a gas chromatograph, with columns of low polarity silicone stationary

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phases (UCW98 and OV-1), became available several years later. By gas chromatography on these columns, the constitution of the inhibitory principle was revealed (Douglas & Francis 1977). It consisted of a complex mixture of mostly homologous 2,4-diketones, derived from the condensation of a molecule of a fatty acid with a molecule of acetone, presumably by an enzymatic mechanism. They ranged from C_{13} to C_{25} in carbon chain length (Douglas et al 1978; Douglas 1991). Although individual 2,4-diketones are well known and are easily synthesized, this lipid class had never previously been identified in animal biochemistry, as the components always occur as a complex mixture in tissue or in urine extracts, which required the resolving power of gas chromatography to separate the components. Since high-pressure liquid chromatography was not then available to us, this technique was not employed. As our investigations were confined to mammals, it is not known if this lipid class occurs in all other forms of animal life.

By virtue of keto-enol tautomerism, 2,4-diketones are potent chelating agents, readily forming, with many divalent and polyvalent metal cations, thermodynamically stable chelates. Such chelates behave in some respects as organic compounds, being soluble in organic solvents, for example. Some chelates may form addition complexes with other organic molecules.

In a preliminary study of the distribution of the 2,4-diketone lipids in rat liver by fractional ultracentrifugation, it was found that the highest amount was in the cytoplasmic fraction, with lesser amounts in the nuclear, microsomal and mitochondrial fractions, in that order (Stotland 1968).

When in the late 1960s, I communicated to Szent-Györgyi our finding regarding the 2,4-diketones, he replied that our results were interesting, but not relevant to his work. Recently, I reviewed Szent-Györgyi's publications concerning retine. A comparison of the recorded physical, chemical and other data about retine with the 2,4-diketone lipid class conglomerate demonstrated their remarkable similarity: their ubiquitous presence in all mammalian tissues and in urine, their infra-red and ultraviolet absorption spectra, denoting the presence of two molecular C=O groups, their solubility in non-aqueous solvents, and their biological activity of an inhibitory nature all concur. In the absence of any evidence to the contrary, I am led to conclude that they are identical.

M. P. Kalapos recently published an overview, with 149 references, of Szent-Györgyi's promine/retine theory of cell division as it was originally proposed in 1965 to its present status (Kalapos 1999). He reviewed the early assumption that retine was a derivative of methylglyoxal, as an inhibitor of cell division, and that the glyoxalase enzyme system, with glutathione as co-enzyme, detoxified methylglyoxal (as retine) to lactate, thus promoting cellular proliferation. The glyoxalase system could be promine. A fine balance between inhibition of cellular division and proliferation was postulated. Kalapos found that, over the years, the theory became less and less acceptable, partly because the intracellular concentration of methylglyoxal and glyoxalase activity did not undergo the expected changes during cell division, the expected relationships of methylglyoxal concentrations and glyoxalase activities between cancerous and normal tissues were not apparent, and retine and promine remained unidentified. It is unfortunate that reference by Kalapos to our publications on the physiological 2,4-diketone lipid class was omitted, for the retine enigma had already been solved. The identification of retine as a 2,4-diketone lipid conglomerate has rendered Szent-Györgyi's promine/retine theory untenable. With modern advances in molecular biology, this theory had seemed too simplistic to have validity. One observation still is viable, namely that the glyoxalase system detoxifies intracellular methylglyoxal and the other α-ketoaldehydes resulting from metabolic activity – a not unworthy function.

There being no publications other than ours concerning this lipid class, its function (or functions) in the life process is unknown. It is of interest that a voluminous literature has accumulated since 1950 (Kovacs 1950; Karady et al 1951) and, perhaps, since even earlier (Haydu et al 1941), about this principle, now identified, which is present in urine and in animal tissues, antagonizes the action of agonists on smooth muscle preparations, and which has other inhibitory properties in-vivo and in-vitro. It is also significant that several non-physiological compounds possessing β-dicarbonyl structures, or a potential for chelate formation through keto-enol tautomerism, exhibit similar inhibitory properties in-vitro as do the physiological 2,4-diketones (Douglas 1993).

Future research may well demonstrate potential uses for the 2,4-diketones in the therapy of cancer (as Szent-Györgyi had hoped) and of inflammatory diseases, such as rheumatoid arthritis, and of psoriasis.

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