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## Biochemical activities of tubercidin 3′, 5′-cyclic monophosphate in rat epididymal adipose tissue*

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ELUCIDATION of the molecular basis for the various actions of adenosine 3′, 5′-cyclic monophosphate (cAMP)† has been assisted greatly by the availability of several types of analogues, particularly those acylated on the $N^6$ and $O^{2'}$ positions,[1–3] and those differing in the base moiety.[4,5] Among the latter is tubercidin 3′, 5′-cyclic monophosphate (cTuMP), first prepared by Hanze[6] from tubercidin (7-deazaadenosine), a nucleoside first isolated from cultures of *Streptomyces tubercidus* by Anzai and Marumo.[7] Tubercidin itself possesses antibiotic, antitumor and antiviral activities, and is highly cytotoxic.[6,9]

cTuMP exhibits several biochemical activities equivalent to those of cAMP. Although cTuMP failed to emulate cAMP as an activator of aged preparations of tryptophan pyrrolase,[8,9] the tubercidin derivative and cAMP were equally active in the glycogen phosphorylase activation assay.[6,10] In addition, cTuMP was hydrolyzed by a partially purified rabbit brain cyclic nucleotide phosphodiesterase (PDE) at a rate three times that of cAMP.[10] It seemed likely therefore that, in certain mammalian systems regulated by cAMP, substitution of a C-H for the $N^7$ of cAMP to form cTuMP might lead to alterations in biological activity. The two experimental situations described below tested this possibility.

Lipolytic activity was tested in a rat epididymal adipocyte system, first described by Mosinger and Vaughan,[11] in which $Ca^{2+}$ and $Mg^{2+}$ are replaced by $Na^+$ in Krebs–Ringer phosphate buffer (pH 7·4) during the collagenase step[12] of cell preparation, and $Na^+$ is the sole cation in the buffer used in all subsequent steps. These changes in the ionic environment of adipocytes apparently permit entry of cAMP and subsequent stimulation of lipolysis,‡ [11] (cf. Table 1), in contrast to the ineffectiveness of cAMP and the potency of $N^6$-monobutyryl adenosine 3′, 5′-cyclic monophosphate and $N^6$, $O^{2'}$-dibutyryl adenosine 3′, 5′-cyclic monophosphate (DBcAMP) when they are used with adipocytes prepared and used in standard Krebs–Ringer bicarbonate or phosphate buffers.‡[3,13] Cells were incubated with various additions for 3 hr at 37°, after which lipolysis was quantified by assay of the glycerol released into the medium.[14] Adipocyte weight was estimated by ester analysis of tissue extracts and equating 3 μequiv. of ester with 1 mg cells.[11] cTuMP, a generous gift from Dr. A. R. Hanze (The Upjohn Co.), and cAMP and DBcAMP (Boehringer-Mannheim) were chromatographically pure [thin-layer chromatography on MN-300 (u.v.) cellulose plates, using ethanol–1 M ammonium acetate (7:3) as solvent].

TABLE 1. LIPOLYTIC ACTIVITY OF CYCLIC NUCLEOTIDES IN ISOLATED RAT EPIDIDYMAL ADIPOCYTES*

| Nucleotide concn. (mM) | Glycerol produced (μmoles produced/g cells in 3 hr) | | |
|---|---|---|---|
| | cTuMP | DBcAMP | cAMP |
| 0·1 | 3·02 | 2·96 | 0·61 |
| 0·25 | 4·64 | 5·42 | 1·74 |
| 0·5 | 6·73 | 7·05 | 2·51 |
| 1·0 | 7·33 | 8·40 | 3·40 |
| 1·5 | 7·88 | 8·20 | 3·60 |

* The value for basal lipolysis was 0·45; for lipolysis in the presence of 1·5 mM tubercidin 5′-P, the value was 0·32.

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† Abbreviations used: tubercidin 3′, 5′-cyclic monophosphate, cTuMP; adenosine 3′, 5′-cyclic monophosphate, cAMP; $N^6$, $O^{2'}$-dibutyryl cAMP, DBcAMP; cyclic nucleotide phosphodiesterase, PDE; nucleoside 3′, 5′-cyclic monophosphates of guanine, inosine and cytosine, cyclic GMP, IMP and CMP.

‡ M. BLECHER, J. T. RO'ANE and P. D. FLYNN, unpublished observations.

TABLE 2. ACTIVITY OF ADIPOSE TISSUE CYCLIC NUCLEOTIDE PHOSPHODIESTERASE WITH ADENOSINE AND TUBERCIDIN 3′, 5′-P

| | cAMP | cTuMP |
|---|---|---|
| $V_{max}$*† | 1·0 | 0·71 |
| $K_m$ (mM)* | 0·15 | 0·24 |

* Determined by a Lineweaver–Burk plot. Means of duplicate experiments.

† Micro-moles of substrate hydrolyzed per mg protein in 30 min.

The effectiveness of cTuMP as a substrate for adipose tissue PDE activity was examined using a partially purified enzyme preparation isolated from rat epididymal fat pads. Tissue homogenates (20–50% in 0·33 M sucrose) were centrifuged at 37,000 *g* at 4° for 30 min. The fluid below the congealed fat cake was then treated with solid ammonium sulfate at pH 7 to obtain the proteins precipitating between 0 and 50 per cent of saturation. The enzyme, dissolved in a solution which was 1·0 mM each with $MgSO_4$ and Tris buffer (pH 7·5), was assayed in a system similar to that of Butcher and Sutherland;[15] $P_i$ was estimated by a modification of the method of Buell *et al.*[16] in which reaction mixtures were incubated at 37° for 30 min prior to the determination of absorbancies at 720 nm.

The results (Table 1) of a representative lipolysis experiment reveal that cTuMP was a far more effective lipolytic agent than was cAMP. Indeed, cTuMP was nearly as effective as DBcAMP, hitherto found to be the most potent lipolytic agent among the cyclic nucleotides in all adipocyte systems.*,[3] As is the case with other 5′-nucleotides, tubercidin 5′-P was not a lipolytic agent. It might be noted parenthetically that cTuMP was also more effective than any of the other purine and pyrimidine 3′-5′-cyclic nucleotides tested.*

A comparison of cTuMP with cAMP as a substrate for a partially purified preparation of adipose tissue PDE revealed that the tubercidin derivative was somewhat less effective than cAMP (Table 2). However, our unpublished experiments with a wide variety of purine and pyrimidine cyclic nucleotides show that, although $V_{max}$ values with cTuMP as substrate were less than those observed with cyclic GMP and cyclic IMP, cTuMP had a greater affinity for PDE than did the purine cyclic nucleotides.* In this connection, it is of interest that a rabbit brain PDE, of a purity similar to that of the present preparation, hydrolyzed cTuMP at a velocity three times that of cAMP; however, alterations in the sugar or phospho moieties of cAMP resulted in a complete loss of the ability to serve as a substrate for PDE or to activate rabbit skeletal muscle phosphorylase b.[10]

The present and past observations indicate therefore that, with one notable exception, the type of base employed in cyclic nucleotides is of less significance for attack by PDE than are the configuration and composition of the 3′, 5′-cyclic phosphate moiety; the exception is cyclic CMP, which is not hydrolyzed by a number of mammalian PDE's.*,[5,15,17] Less clear is the situation regarding the biological activities of these compounds. In these cases it appears that, although the usual 3′, 5′-cyclic phosphodiester structure (as in cAMP) is an absolute requirement for biological activity, no such absolutism applies to the base moiety. In studies to be published elsewhere, we will show that a relatively high degree of specificity, insofar as biological activity is concerned, resides in the structures of the purine and pyrimidine bases.

*Department of Biochemistry,*
*Schools of Medicine and Dentistry,*
*Georgetown University,*
*Washington, D.C. 20007, U.S.A.*

MELVIN BLECHER
J. T. RO'ANE
P. D. FLYNN

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## Lysosomal enzyme levels in the blood of arthritic rats

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ACID hydrolases have been suggested as possible chemical mediators of inflammation associated with rheumatoid arthritis.[1] Adjuvant induced arthritis in rats is often used as a model for the human disease. In this investigation *N*-acetyl-β-D-glucosaminidase (EC 3.2.1.30), acid phosphatase (EC 3.1.3.2) and β-glucuronidase (EC 3.2.1.3) have been determined in the serum or plasma of rats treated with mycobacterial adjuvant. These enzymes are normally regarded as lysosomal in origin.

*Experimental*

Arthritis was induced in Wistar strain rats (females, 160–170 g; males, 250–300 g) by methods previously described.[2] In one experiment the adjuvant was introduced into the right hind foot pad. In a second experiment the adjuvant was injected into the tail. Human strains (C, DT and PN) of tubercle bacilli were kindly supplied by the Ministry of Agriculture Veterinary Laboratories, Weybridge, Surrey. Blood was removed from the aorta of rats under deep anaesthia induced by a $CO_2$–$O_2$ mixture (1 : 1, v/v) delivered from a Boyle's apparatus. In a second experiment anaesthia was induced by ether. In one experiment the diameter of the ankle joint (right hind foot) was measured in order to assess inflammation. In the second experiment, where the adjuvant was injected into the tail, the mean diameter of the ankle joints of both hind legs was calculated. *N*-acetyl-β-D-glucosaminidase,[3] acid phosphatase[4] and β-glucuronidase[4] were determined in serum or plasma. In the case of β-glucuronidase it was found necessary to incubate the serum with the substrate for 18 hr.

*Results and discussion*

The results of the two experiments are shown in Figs. 1 and 2. In the first experiment the adjuvant was injected into the feet of female rats. Acid phosphatase and β-glucuronidase activities in the experimental serums are shown in Fig. 1. Control acid phosphatase and β-glucuronidase values (36 rats) were found to lie in the ranges 1·3–1·7 μM *p*-nitrophenol/ml serum/hr for acid phosphatase and 0·2–0·3 μM phenolphthalein/ml serum/hr for β-glucuronidase. Values determined in the experimental serums outside of these figures were regarded as abnormal. The mean diameter for the controls of the right hind ankle joint was found to be 6·8 ± 0·1 mm. The results (Fig. 1) show that there was some elevation