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Steiner A L, Parker C W & Kipnis D M. Radioimmunoassay for cyclic nucleotides. I. Preparation of antibodies and iodinated cyclic nucleotides. *J. Biol. Chem.* 247:1106-13, 1972.
[Divs. Metab. and Immunol., Dept. Med., Washington Univ. Sch. Med., St. Louis, MO]

Sensitive and specific radioimmunoassays for adenosine-3',5'-cyclic monophosphate (cyclic AMP) and guanosine-3',5'-cyclic monophosphate (cyclic GMP) have been developed. The radioimmunoassay systems are based upon competition of the cyclic nucleotide with a labeled cyclic nucleotide derivative of high specific activity for binding sites on an antibody specific for the cyclic nucleotide. [The *SCI*® indicates that this paper has been cited in over 1,360 publications since 1972.]

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In 1967 I joined Kipnis's laboratory at the Washington University School of Medicine as a postdoctoral fellow. At that time, there was a growing awareness of the universal role of adenosine-3',5'-cyclic monophosphate (cyclic AMP) as a second messenger for hormones.[1] To show that cyclic AMP was a second messenger in hormone action, it was necessary to document a change in the concentration of the cyclic nucleotide in a tissue extract after addition of the hormone. The assays utilized for measuring cyclic AMP at that time were insensitive and required extensive purification of the tissue extract.

I was having difficulty obtaining reproducible results after over 18 months of effort to set up a cyclic AMP assay. In desperation, I decided to try to make antibodies to cyclic AMP. We followed the work of Falbriard *et al.*[2] for making butyryl derivatives of cyclic AMP. Our thinking was that the most important antigenic sites on the cyclic nucleotide would be the 3'5'-cyclic phosphate ring and the charges on the purine nucleus. We felt the 2'O succinyl site on cyclic AMP would be the least important antigenically and made a 2'O succinyl derivative that we linked to a protein. We immunized rabbits with the conjugate and additionally made a 2'O succinyl tyrosine methyl-ester derivative of cyclic AMP (ScAMPTME) that we could iodinate. These reactions were run in organic solvents and had poor yields.

Our initial iodination of [^{125}I]ScAMPTME yielded on purification two sharp peaks from a Sephadex G-10 column with a broad trailing peak. You can imagine my excitement when I came back to the gamma counter and found that the rabbit antiserum bound virtually all of the ^{125}I from the third peak. Within a week, I established the conditions for the radioimmunoassay and found that I could measure cyclic AMP in rat-tissue extracts without purification and with picomolar sensitivity. Some of the antisera distinguished cyclic AMP from ATP by a factor of 10^6.[3] We could process hundreds of tissue samples weekly. Shortly thereafter we developed a similar radioimmunoassay for guanosine-3',5'-cyclic monophosphate (cyclic GMP).

The finding by Cailla and Delaage[4] that succinyl derivatives of cyclic AMP could be made in water led to the development of radioimmunoassays that derivatize the cyclic nucleotides at the 2'O position prior to assay. These radioimmunoassays detect femtomolar amounts of the cyclic nucleotides and are readily available commercially.

Our radioimmunoassays for the cyclic nucleotide revolutionized measurement of cyclic AMP and cyclic GMP in tissues and body fluids and allowed many investigators to work in the area of second-messenger function. At the same time, Gilman[5] developed a sensitive and specific assay for cyclic AMP utilizing the receptor subunit of cyclic AMP protein kinase as the binding protein. By the 1970s, investigators could choose several excellent methods for measuring cyclic nucleotides in their work. These technologies helped to catalyze the understanding of the mode of action of a wide variety of hormones acting via cyclic AMP to control cellular metabolism and growth.

1. **Butcher R W, Ho R J, Meng H C & Sutherland E W.** Adenosine-3',5'-monophosphate in biological materials. II. The measurement of adenosine 3',5'-monophosphate in tissues and the role of the cyclic nucleotide in the lipolytic response of fat to epinephrine. *J. Biol. Chem.* 240:4515-23, 1965. (Cited 600 times.)
2. **Falbriard J G, Posternak T & Sutherland E W.** Preparation of derivatives of adenosine 3',5'-phosphate. *Biochim. Biophys. Acta* 148:99-105, 1967. (Cited 120 times.)
3. **Steiner A L, Kipnis D M, Utiger R & Parker C W.** Radioimmunoassay for the measurement of adenosine 3',5'-cyclic phosphate. *Proc. Nat. Acad. Sci. US* 64:367-73, 1969. (Cited 690 times.)
4. **Cailla H & Delaage M.** Succinyl derivatives of adenosine 3',5'-cyclic monophosphate: synthesis and purification. *Anal. Biochem.* 48:62-72, 1972.
5. **Gilman A G.** A protein binding assay for adenosine 3',5'-cyclic monophosphate. *Proc. Nat. Acad. Sci. US* 67:305-12, 1970. (Cited 4,065 times.)