CC/NUMBER 28
JULY 12, 1993

This Week's Citation Classic®

Sattin A & Rall T W. The effect of adenosine and adenine nucleotides on the cyclic adenosine 3',5'-phosphate content of guinea pig cerebral cortex slices. *Mol. Pharmacol.* **6**:13-23. 1970.
[Departments of Pharmacology and Psychiatry, Case-Western Reserve University. Cleveland. OH1

This paper described the induction of large accumulations of cyclic AMP in brain slices by adenosine and precursor adenine nucleotides. It also documented surmountable (competitive) inhibition of adenosine's effects by the methylxanthines, theophylline and caffeine. While evidence for alternative explanations was sought, we concluded that there existed receptors for adenosine linked to cyclic AMP synthesis and that the methylxanthines antagonized these receptors. [The *SCI*® indicates that this paper has been cited in more than 855 publications.]

Methylxanthines as Antagonists of Adenosine Receptors

Albert Sattin
Department of Psychiatry and Biobehavioral Sciences
Sepulveda VA Medical Center and
UCLA School of Medicine
Sepulveda, CA 91343

As reviewed by Theodore W. Rall,[1] this work evolved from observations that led to the discovery of cyclic AMP and its regulatory role. These included the serendipitous discovery that methylxanthines promoted the accumulation of the "heat-stable factor" (cyclic AMP) responsible for the activation of glycogen phosphorylase by epinephrine and glucagon in liver homogenates.[2] Eventually, the methylxanthines became firmly established as inhibitors of cyclic nucleotide phosphodiesterases (PDEs), and they were extensively used to investigate hormonal actions that might be mediated by cyclic AMP.

This paper emanated specifically from the work of my collaborator, Rail (now at the University of Virginia), who with Shiro Kakiuchi (deceased) was investigating the capacity of candidate neurotransmitters to increase the cyclic AMP content of brain slices. In those studies, the stimulatory effects of norepinephrine and histamine were enhanced, as expected, by theophylline.3 While that work was in progress in 1965, I was in the laboratory of Henry McIlwain (deceased) in London. At my suggestion, Kakiuchi spent three months in London in order to explore the effects of electrical pulses on cyclic AMP in brain slices. The analyses, carried out by Rail in Cleveland, fortunately included experiments done with and without theophylline. The astonishing results of five minutes of electrical field stimulation were not only a 10-fold increase in cyclic AMP, but an 85 percent reduction of this effect by theophylline.[4] Upon my return to Cleveland, Rail and I searched crude brain extracts for stimulating factors released by the pulses. Happily, such extracts not only increased cyclic AMP 20-fold, but those responses were virtually obliterated by theophylline. However, we were puzzled that extracts of muscle were even more potent, and that ion-exchange chromatography yielded four distinct, active fractions.[5] Rail's accurate guess that adenosine and its nucleotides could produce this pattern of results obviated the rediscovery of adenosine, and the experiments described in this paper ensued.

In retrospect, previous observations of antagonistic interactions between adenosine and methylxanthines might have suggested receptor mechanisms.[1] We, however, through the historical sequence described above, were conditioned to the receptor concept and were subsequently forced to diverge from the dogma of methylxanthines as PDE inhibitors. Thus, the proposed existence of adenosine receptors was inseparable from our simultaneous observation of this new effect of the methylxanthines. Previous explanations of methylxanthine effects, e.g., direct mobilization of calcium ions and inhibition of PDE, have been rejected because the human doses required for those effects exceeded normal or therapeutic doses.[1]

Most often cited as the origin of the adenosine receptor concept,[6] citation of our *Classic* paper for the simultaneous origin of the methylxanthine mechanism has usually been overlooked. It is now widely accepted that the behavioral and cardiovascular effects of caffeine and theophylline result from their ability to block the actions of ambient adenosine.

1. **Rall T** W. Evolution of the mechanism of action of methylxanthtnes: from calcium mobilizers to antagonists of adenosine receptors. *Pharmacologist* 24:277-87, 1982.
2. **Bcrthet J, Sutherland E W & Rall** T **W.** The assay of glucagon and epinephrine with use of liver homogenates. J. *Biol. Chem.* 229:351-61. 1957.
3. **Kakluchi S & Rail T W.** Studies on adenosine 3',5'-phosphate in rabbit cerebral cortex. *Mol. Pharmacol.* 4:379-88. 1968. (Cited 320 times.)
4. **Kakiuchi S, Rall T W & Mcllwain H.** The effect of electrical stimulation upon the accumulation of adenosine 3',5'-phosphate in isolated cerebral tissue. *J. Neurochem.* 16:485-91, 1969. (Cited 270 times.)
5. **Rall T W & Sattin A.** Factors influencing the accumulation of cyclic AMP in brain tissue. *Advan. Biochem. Psychopharm.* 3:13-33. 1970.
6. **Bruns R F.** Adenosine receptors: roles and pharmacology. *Ann. SYAcad. Sci.* 603:211-26. 1990.
Received April 29. 1993