Inhibition of Rat Liver Adenyl Cyclase by Adenosine and Adenine Nucleotides[1]

In the course of experiments designed to measure the cyclase activity of rat liver fractions, we noted that the increase in adenosine-3′, 5′-monophosphate (cyclic AMP) production induced by fluoride was accompanied by a decrease in the production of other derivatives of ATP (inosine, hypoxanthine and adenosine). Since ADP, AMP and other nucleotides, in the presence of fluoride, inhibit bacterial[2] and adrenal cyclase[3], we investigated the possibility that these substances may inhibit also hepatic cyclase activity.

Material and methods. The cyclase assay was based on a modification of the method of WEISS and COSTA[4] in which cyclic AMP was purified by paper chromatography instead of column chromatography and $BaSO_4$ precipitation. Livers of male Sprague-Dawley rats were homogenized[5], the pellet obtained after centrifugation in 0.25 *M* sucrose at 2000 *g* for 30 min was suspended in 0.02 *M* glycylglycine buffer, pH 7.7, containing 1 m*M* $MgSO_4$, recentrifuged at 2000 *g* for 10 min, resuspended in glycylglycine buffer (100 mg/2 ml) and kept at 4 °C. 10 µl of this suspension were added to each sample just prior to incubation. Its protein content was determined using the method of LOWRY et al.[6]. Each 0.06 ml sample contained ATP-α-^{32}P (30–50 mc/mmole, Schwarz BioResearch), 1.5 m*M*; caffeine, 50 m*M*; $MgCl_2$, 5 m*M*; *tris*-HCl, 25 m*M*, pH 7.7 at 30 °C; crystalline bovine serum albumin (Sigma), 1%; and NaF, 10 m*M*. Adenosine and other substances (Sigma) were added in amounts calculated to produce a 1 m*M* final concentration. All tubes were incubated at 30 °C for 30 min. The reaction was terminated by heating the tubes in boiling water for 3 min. Just prior to heating, 20 µl of a solution containing 20 µg of cyclic AMP, 20 µg of ATP and a trace of ^{14}C-cyclic AMP (5000 cpm) were added to each tube to check the recovery of cyclic AMP in the purification system. After centrifugation, the supernatant was purified by descending chromatography using Whatman No. 40 filter paper. Preliminary experiments indicated that 4 separate solvent systems were needed to obtain the desired purity of cyclic AMP (butanol-glacial acetic acid-water, 5:2:3; isopropanol-glacial acetic acid-water, 6:3:1; ethanol-0.1 *M* boric acid, 3.5:1 and isopropanol-conc. ammonium hydroxide-water, 7:1:2). After the final separation, ^{32}P and ^{14}C in the cyclic AMP spot were measured simultaneously by liquid scintillation. The recovery of cyclic AMP was 15–30%.

Results. The Table summarizes the results of 6 experiments showing that cyclase activity was decreased consistently by 60%, 50% and 40% in the presence of adenosine, of AMP and of ADP, respectively. Adenine, hypoxanthine, inosine and IMP had no significant effect. In one preliminary experiment, ribose-5-P (used in place of ribose-1-P for reasons of economy) had a slight inhibitory effect.

Discussion. The mechanism of cyclase inhibition by nucleotides has not been determined. In mouse adrenal tumor[3], ADP was found to be a more effective inhibitor than AMP and the effect of both nucleotides varied with the concentration of Mg^{++}. Thus, the fact that, in our experiments, AMP was more effective than ADP, may be explained by differences in the tissue used and in the concentration of Mg^{++}, which was 5 m*M* in our experiments and varied between 3.1 and 5.6 m*M* in those of TAUNTON et al.[3]. The inhibitory effect of adenosine on cyclase activity may explain the observation that adenosine has an insulin-like effect on lipolysis in vitro[7] and that a 0.08 m*M* concentration of adenosine is sufficient to inhibit the lipolytic action of noradrenaline in adipose tissue[8]. Indeed this effect was ascribed to a postulated inhibition of cyclase and of cyclic AMP-stimulated lipolysis[8]. Our experiments provided direct evidence that a 0.08 m*M* concentration of adenosine can cause a 40%-inhibition of rat liver adenyl cyclase in the presence of fluoride (Table, Experiment 7). One of the explanations for the stimulatory effect of fluoride on cyclase activity is that it decreases the breakdown of ATP, thus preserving substrate for the enzymatic reaction. One may add that this would result also in a decreased formation of derivatives capable of acting as cyclase inhibitors. Although further experiments are necessary to determine the significance of these findings, we suggest that, by inhibiting cyclase activity, adenosine and other nucleotides may play a role in regulating the action of the cyclase-dependent hormones[9].

Effect of nucleotides and their metabolites on the cyclase activity of rat liver particulate fractions obtained by centrifugation at 2000 *g*

Additions	(Cyclic AMP formed, nmoles/g protein/30 min) Experiment 1	2	3	4	5	6	7[a]	
None				62	48			
Fluoride	400	630	427	262	186	269	(495)	
F⁻, ADP	243	374	261					$P<0.05$
F⁻, AMP	208	248	204	147	113		(346)	$P<0.025$
F⁻, Adenosine		247	168	110	95	112	(306)	$P<0.025$
F⁻, Adenine		611	367					
F⁻, Hypoxanthine						260		
F⁻, Inosine						246		
F⁻, IMP						279		
F⁻, Ribose-5-P						222		
Protein (mg/tube)	0.24	0.25	0.30	0.18	0.23	0.35	0.27	

All values represent the average of 3 determinations. In each experiment, values obtained in the presence of fluoride and additives were treated as a pair in calculating the *P* values. [a] The concentration of adenosine and AMP was 0.083 m*M*. These results were not included in the calculation of *P* values.

Riassunto. L'attività adenilciclasica delle frazioni subcellulari di fegato di ratto fu inibita dall'adenosina, dall'AMP e dall'ADP (1 m*M*) del 60, 50 e 40%. Alla concentrazione di 0.08 m*M*, l'adenosina e l'AMP inibirono l'attività ciclasica del 40 e del 30%. L'IMP, l'inosina, l'adenina e l'ipoxantina furono senza effetto.

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