Mechanism of Prostaglandin Biosynthesis. III. Catecholamines and Serotonin as Coenzymes

Sir:

The biosynthesis of prostaglandin-E_1 (PGE_1) from 8,11,14-eicosatrienoic acid by ovine seminal vesicle preparations was shown to require reduced glutathione (GSH) and hydroquinone (HQ),[1] but the roles of the latter compounds in this oxidative cyclization reaction have not been clearly defined. Accordingly to Nugteren, *et al.*,[2] 2.4 mol of GSH was consumed per mole of fatty acid metabolized and HQ was required as an antioxidant. In this communication, experimental evidence is presented to show that catecholamines and serotonin can act as coenzymes by providing the reducing equivalents for prostaglandin synthesis.

A wide variety of compounds was observed to replace the HQ in this oxidative cyclization reaction.[1] This raises the question as to the identity of the natural coenzyme. As catechol could substitute for HQ, albeit poorly, in the bovine seminal vesicle system (BSVM), we suspected that the natural coenzyme may be the catecholamines. It was found that both L-norepinephrine and L-epinephrine effectively supported prostaglandin formation whereas dopamine and L-3,4-dihydroxyphenylalanine were less effective. More striking, however, is the finding that the ratio of prostaglandins can be altered during biosynthesis. Table I shows

Table I. Effect of Coenzymes on Prostaglandin Synthesis

	μmol			
	PGA[a]	PGD	PGE	PGF
HQ (pH 7.5)	1.3	3.0	11.0	1.0
HQ (pH 9)	2.6	0.7	1.7	
L-Epinephrine (pH 9)	1.4	2.9	13.4	11.5
Serotonin (pH 9)	2.4	6.3[b]	18.3	4.4
5-Hydroxyindole-3-acetic acid (pH 9)		3.8	11.8	2.2

[a] PGA was formed in the work-up. [b] In some experiments, yields of PGD were as high as 10 μmol. The reaction mixture consisted of 65 μmol of arachidonic acid (5 μCi of tritium), 1.64 mmol of coenzyme, and 1 g of BSVM in a total volume of 100 ml of 0.05 *M* Tris buffer at the pH indicated. After incubation for 1 hr at 37°, the contents were acidified to pH 2.0 and extracted with ethyl acetate. The ethyl acetate residue was chromatographed over a silicic acid column and the radioactive peaks were combined and assayed.

that the ratio of 11-dehydro-PGF[3] (PGD), PGE, and PGF using HQ was 3:11:1, respectively, whereas this ratio was changed to 3:13:12 using L-epinephrine. Because a substantial amount of tryptophan was found in the supernatant fraction of bovine seminal vesicles, we examined the hydroxyindoles as possible coenzymes. Both serotonin and 5-hydroxyindole-3-acetic acid (5-OH-IAA) were capable of supporting prostalgandin synthesis. While L-epinephrine enhanced PGF formation, serotonin favored the formation of PGD.

To confirm that the reducing equivalents in prostaglandin formation are derived from these compounds, we selected 5-OH-IAA as a model for stoichiometric studies. When the disappearance of 5-OH-IAA-^{14}C was correlated with the amount of prostaglandin formed, it was noted that approximately 2 mol of 5-OH-IAA was consumed per mole of prostaglandin formed (Table II). When GSH was included

Table II. Stoichiometry of 5-OH-IAA Consumption during Prostaglandin Formation[a]

Additions	μmol of Total PG formed (A)	μmol of 5-OH-IAA used (B)	Ratio B/A
5-OH-IAA	1.4	2.9	2.1
5-OH-IAA + GSH	2.4	0.49	0.2

[a] The system contained 3.3 μmol of arachidonic acid (5 μCi of tritium for PG assay), 16.2 μmol of 5-OH-IAA (2 μCi of ^{14}C for 5-OH-IAA assay), 13 μmol of GSH (where indicated), and 100 mg of BSVM in 10 ml of 0.05 *M* Tris buffer (pH 9.0). The mixture was incubated at 37° for 30 min and worked up as in Table I. The prostaglandins were eluted off the column with a gradient system of benzene–ethyl acetate. 5-OH-IAA was eluted from the same column with a 6:4 mixture of ethyl acetate–benzene.

in the incubation mixture, the amount of 5-OH-IAA utilized decreased to a value of 0.2 per mol of prostaglandin formed. This result clearly shows that 5-OH-IAA is the source of reducing equivalents. Furthermore, GSH appears to reduce the oxidized form of 5-OH-IAA back to the reduced form. The effect of GSH was reported to be specific and could not be replaced by other SH reagents.[2] Thus, it is possible that this reduction is enzymic or that GSH also behaves as an allosteric effector.

Catecholamines, serotonin, and prostaglandins are all widely distributed throughout various tissues. One could speculate that catecholamines and serotonin may be the natural coenzymes in regulating the *in vivo* biosynthesis of prostaglandins. Furthermore, it may be that one of the important physiological roles of these hormones is to furnish reducing equivalents for a variety of oxygenation reactions.

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Dichloromethyladamantanes. Formation *via* Carbene Insertion and Hydrolytic Rearrangement to Homoadamantanone

Sir:

We wish to report the rearrangement of 1-dichloromethyladamantane (**1**) to homoadamantanone (**2**) in the presence of hot phosphoric acid. We also wish to report the extraordinarily efficient dichlorocarbene insertion reaction with adamantanes giving 1-dichloromethyladamantane (**1**) exclusively and in nearly quantitative yield. It was the first successful dichlorocarbene insertion into a saturated hydrocarbon.