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EFFECT OF OLIGOMERIC DERIVATIVES OF PROSTAGLANDIN B_1 ON OXIDATIVE PHOSPHORYLATION AND THEIR Ca^{2+} IONOPHORETIC ACTIVITY

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Summary: Dimers, trimers and tetramers of 15-dehydro-PGB_1 and of 16,16'-dimethyl-15-dehydro-PGB_1 have been synthesized and their effect on mitochondrial function evaluated. The trimers and tetramers, and to a lesser extent the dimers, of both series, protected isolated mitochondria from the loss of phosphorylating capacity during in vitro incubation. The monomers were inactive. The trimers and tetramers inhibited between 40 and 50% the F_1F_0-ATPase of submitochondrial particles. All of the oligomers, but not the monomers, had Ca^{2+} ionophoretic activity with isolated mitochondria. These activities are qualitatively similar to that reported for the oligomeric mixture of 15-dehydro-PGB_1, termed PGB_X.

Polis et al (1) reported the preparation of a mixture, which was termed PGB_X, of products of a base catalyzed oligomerization of prostaglandin B_1; the mean molecular weight of the substances in PGB_X was about 2200. The oligomeric mixture has a number of interesting biological activities, including its ability to protect isolated rat liver mitochondria against loss of oxidative phosphorylation during in vitro incubation (1) and Ca^{2+} ionophoretic activity with mitochondria (2), sarcoplasmic reticulum (2) and liposomes (3). It has been reported that in vivo PGB_X protects animals subjected to myocardial (4) or cerebral ischemia (5) or to hypoxia (6). The complexity of the mixture in PGB_X samples has precluded the isolation and identification of an active compound or compounds.

Careful control of reaction conditions has permitted the preparation of oligomeric mixtures from both 15-dehydro-PGB_1 (7) and 16,16'-dimethyl-15-dehydro-PGB_1 (16.16'-diMe-PGB_1) from which the dimer, trimer and tetramer components were isolated. Oligomeric mixtures derived from 15-dehydro-

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PGB_1, via a Michael addition pathway, contain oligomers of both single-addition (C-10 to C-13' or C-14') and a double-addition where C-16 adds to C-14' with the further addition of the C-14 to C-13', or C-16 to C-13' and addition of C-14 to C-14', in both cases forming a cyclopentanone ring (7). Mixtures derived from 16,16'-diMe-PGB_1 were much less complex since the C-16 derived double-addition pathway was eliminated and the C-10 to C-13' bond formation was favored over C-10 to C-14' by the increased steric hinderance at C-16. For example, six dimers were obtained from 15-dehydro-PGB_1 and only three from 16,16'-diMe-PGB_1 (7).

This report describes the ability of these oligomeric derivatives of PGB_1 to protect isolated mitochondria from loss of oxidative phosphorylation and their activity as Ca^{2+} ionophores.

MATERIALS AND METHODS: Oligomeric mixtures of the general formula $(C_{20}H_{30}O_4)_n$, where n = 1-4, were prepared by treatment of 15-dehydro-PGB_1 with 0.05 M ethanolic KOH at room temperature to about 50 percent conversion (7). This mixture was separated by size exclusion chromatography on Sephadex LH-20, using methanol as the carrier solvent, into dimer (n = 2, MW = 668), trimer (n = 3, MW = 1002) and tetramer (n = 4, MW = 1336) components. Treatment of 16,16'-dimethyl- 15-dehydro-PGB_1 under similar reaction conditions resulted in conversion to an oligomeric mixture of the formula $(C_{22}H_{34}O_4)_n$, where n = 1-4 monomer units. This mixture was also readily separable into dimer (n = 2, MW = 724), trimer (n = 3, MW = 1086) and tetramer (n = 4, MW = 1448) components. Each component was dissolved in 95% ethanol (10 mg/ml) and added to the indicated final concentration in the various assays.

All chemicals used were reagent grade or of high purity. Ruthenium red (Sigma Chemical Co.) (8) and murexide (Sigma Chemical Co.) (9) were recrystallized prior to use.

Male Wistar rats weighing from 150 to 200 gm were sacrificed by cervical dislocation and decapitation. Livers were rapidly dissected and immersed in approximately 50 ml of cold 0.25M sucrose, 10 mM Tris-HCl, pH 7.6, in ice. Mitochondria were isolated essentially as described by Hogeboom et. al. (10) and always used within 3 hours after isolation. Submitochondrial particles were prepared by a modification of described procedures (11). Protein was determined by the Biuret method (12). F_1F_0-ATPase activity of submitochondrial particles was measured spectrophotometrically as the oxidation of NADH coupled to the production of ADP via pyruvate kinase and lactate dehydrogenase (13). The oligomers were added immediately prior to ATP. NADH oxidation measured in an Aminco DW-2 spectrophotometer in dual mode, at 340-550 nm.

Oxygen consumption was measured with a YSI 5331 oxygen electrode (Yellow Springs Instrument Company, Inc.), in conjunction with a Gilson K-IC oxygraph. The reaction compartment was stirred constantly. The state 3 respiration was measured following addition of ADP. The respiratory control index for the mitochondrial preparations was between 6 and 8. Mitochondrial Ca^{2+} movements were measured spectrophotometrically using the metallochrome indicator Murexide, 75 uM, in an Aminco DW-2 spectrophotometer

in dual mode, at 535-507 nm (2). The sequence of addition was zero time, mitochondria; one minute, Ca^{2+}; 2.5 to 3 minutes, ruthenuim red to block recycling of released Ca^{2+}, and at about 4 minutes, the PGB_1 oligomer.

RESULTS AND DISCUSSION: The effect of the 16,16'-diMe-PGB_1 oligomers on stabilizing oxidative phosphorylation of rat liver mitochondria was determined by preincubating mitochondria at 30°C with and without the derivative in the absence of ADP. At the times indicated in Figure 1 an aliquot was transferred to the oxygraph cell for measurement of respiration. After a 1 to 2 minute incubation, ADP was added and state 3 (presence of ADP) and state 4 (after phosphorylation of ADP) respiratory rates were determined. The state 3 respiratory rate of the mitochondria decreased with time reaching a minimum value after approximately 30 to 40 minutes. Loss of

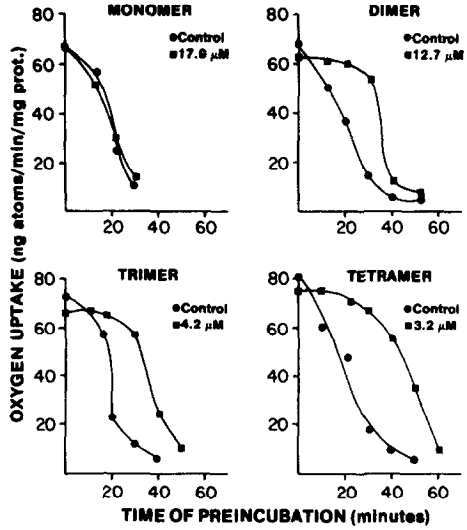

Figure 1. Protective effect of oligomers of 16,16'-dimethyl-15-dehydro PGB_1 on State 3 respiration during preincubation of isolated rat liver mitochondria. The preincubation reaction mixture contained 100 mM KCl, 10 mM MOPS, pH 7.4 7 mM $MgCl_2$, 14 mM phosphate buffer, 20 mM α-ketoglutarate, 4.0 mg mitochondrial protein/ml and the indicated concentration of PGB_1 oligomer; temp: 30°C. The mitochondria were preincubated for the times indicated then an aliquot (1.7 ml) of the incubation medium was transferred to the reaction vessel (30°C) for measurement of respiratory activity as described in Methods. After approximately 1 minute, 340 nmoles of ADP was added and the rate of state 3 respiration was determined. After an appropriate time (2 to 3 minutes or after respiration returned to state 4), 1 uM FCCP (carbonylcyanide-p-trifluoro-methoy phenylhydrazone) was added and uncoupler stimulated respiration was recorded.

state 3 respiration during in vitro aging has been observed by others (14) and occurs concurrently with loss of oxidative phosphorylation and a variety of other changes. The presence of the dimer, trimer and tetramer of 16,16'-diMe-PGB_1 during the preincubation period delayed the decay of state 3 respiration. The monomer had no protective effect even at a concentration four times higher than the tetramer. Similar results were observed using the dimer, trimer and tetramer of 15-dehydro-PGB_1, as well as with PGB_x, the mixture of higher oligomeric forms of 15-dehydro-PGB_1. In the absence of the PGB_1 derivatives the uncoupler (FCCP) stimulated respiration decreased in the same time period as state 3 respiration; the derivatives also delayed this loss. Assaying oxidative phosphorylation by measurement of phosphate incorporation into ATP permitted measurements of dose response curves of the derivatives and the effect of other compounds. Maximum protection was exerted in the range of 3 to 6 uM of each derivative; the amount of oligomer required for protection, however, was dependent on the mitochondrial protein concentration with maximum protection at about 2-4 nmoles of derivative/mg mitochondrial protein. EGTA, serum albumin and other prostaglandins had no protective effect; ATP in the preincubation medium protected the mitochondria. At the concentration of derivatives required to demonstrate a protective effect on the preincubated mitochondria, there was no significant effect on state 3, state 4 or uncoupler stimulated respiration of mitochondria not preincubated. At concentrations six to ten fold higher, however, there was a stimulation of state 4, an inhibition of state 3 and a inhibition of uncoupler stimulated respiration.

The trimer and tetramer of 15-dehydro-PGB_1 and of 16,16'-diMe-PGB_1 inhibited F_1F_0-ATPase of submitochondrial particles (Table 1); the results are presented for 0.57 uM of each derivative but each was tested at higher and lower concentrations. The monomer and dimer had no inhibitory activity even at 38 uM. The maximum percent inhibition by the trimer and tetramer of both series was about 50-55 percent. PGB_x also inhibited the enzyme maximally about 50% (15). The concentration of derivatives required for

Table I. Effect of PGB_1 Oligomers on the F_1F_O-ATPase Activity of Rat Liver Submitochondrial Particles

	ATPase Activity	
PGB_1 Derivative	15-dehydro-PGB_1	16,16'-diME-PGB_1
	nmoles ATP hydrolyzed/min/mg protein	
None	0.77 ± 0.01	
Monomer	0.72 ± 0.01 (6)	0.73 ± 0.02 (5)
Dimer	0.72 ± 0.01 (6)	0.77 ± 0.00 (0)
Trimer	0.51 ± 0.01 (34)	0.50 ± 0.01 (35)
Tetramer	0.35 ± 0.01 (55)	0.40 ± 0.01 (48)

ATP hydrolysis measured as described in Methods. The reaction mixture contained 100 mM KCl, 20 mM MOPS, pH 7.6, 5 mM KCN, 3 mM $MgSO_4$, 0.4 mM phospho-enol-pyruvate, 0.3 mM NADH, 15 IU pyruvate kinase, 15 IU lactate dehydrogenase, and 0.1 mg protein of submitochondrial particles in a final volume of 1.0 ml. PGB_1 derivatives (0.57 uM) added immediately before initiation of reaction with 3 mM ATP. Values are mean ± S.E.M. of duplicate samples from three separate experiments. Numbers in parenthesis indicate percent inhibition.

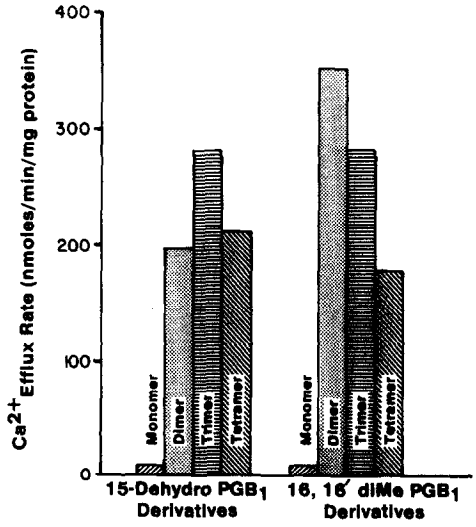

Figure 2: Ca^{2+} efflux from mitochondria mediated by oligomers of 15-dehydro-PGB_1 and 16,16'-dimethyl-15-dehydro-PGB_1. Reaction mixture contained 0.125 M KCl, 5mM Tris-HCl; pH 7.6, 5 mM Na^+ acetate, 5 mM glutamate-malate malate-Tris, pH 7.6, 75 uM Murexide and rat liver mitochondria, 1 mg protein/ml in 3 ml; temp. 25°C. Mitochondria were incubated for 2 minutes and then loaded with Ca^{2+}, 75 nmoles/mg protein. After 2 minutes, 400 nM ruthenium red was added and one minute later the PGB_1 derivative (9.2 uM) was added. Spectrophotometric Ca^{2+} measurements were conducted as described in Methods. Initial Ca^{2+} efflux rates were determined from the slope after addition of PGB_1 derivative.

maximum inhibition of F_1F_0-ATPase was lower than that observed for protection of oxidative phosphorylation.

The oligomeric derivatives of PGB_1 stimulated the release of Ca^{2+} from mitochondria (Figure 2) but the monomers were inactive. This activity of the derivatives was equal to or greater than that reported for PGB_x (2). The release of Ca^{2+} was not due to a disruption of the mitochondria by the derivatives. Studies of their ability to transfer Ca^{2+} to an organic phase demonstrate that they function as Ca^{2+} ionophores (16).

The results demonstrate that the oligomerization of prostaglandin B_1, with the formation of dimers, trimers and tetramers, leads to a major change in the biological properties of the prostaglandin. In addition, the various activities reported for PGB_x are apparently not due to a single component of PGB_x but rather represent the activities of a class of oligomeric forms of PGB_1. It has been reported that in vivo PGB_x protects tissues against damage due to ischemia (4,5,6). It will be of value to determine if these structurally defined derivatives have a similar effect.

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