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Inactivation of Prostaglandin H Synthase and Prostacyclin Synthase by Phenylbutazone

Requirement for Peroxidative Metabolism

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SUMMARY

Phenylbutazone (PB), a nonsteroidal anti-inflammatory drug, is an efficient reducing cofactor for the peroxidase activity of prostaglandin H synthase (PHS). Most reducing cofactors for the peroxidase protect PHS and prostacyclin synthase from inactivation by hydroperoxides. PB, however, does not protect these enzymes, but rather augments their hydroperoxide-dependent inactivation. Using ram seminal vesicle microsomes as a source of PHS and prostacyclin synthase, we have examined the interaction of PB and exogenous hydroperoxides. Chromatographic analysis of the metabolism of ^{14}C-labeled arachidonic acid in this system revealed that PB-dependent inactivation of PHS is markedly increased in the presence of 100 μM H_2O_2. This inactivation is a linear function of PB concentration between 10 and 250 μM, with a half-maximal effect in this range at about 100 μM PB. Prostacyclin synthase is even more sensitive to inactivation by the combined PB and H_2O_2 treatment, with a corresponding half-maximal effect at PB concentrations near 25 μM. This PB- and H_2O_2-dependent inactivation is demonstrable whether PGH_2 is generated *in situ* from arachidonic acid or is added exogenously, supporting a direct effect of the treatment on prostacyclin synthase. As PB undergoes peroxide-dependent co-oxygenation catalyzed by PHS, we propose that it is an oxygenated derivative of PB, rather than the parent compound, which is responsible for the inactivation of PHS and prostacyclin synthase. Nafazatrom, a competitive inhibitor of PB co-oxygenation, blocks the effects of the PB and H_2O_2 treatment, supporting our proposal.

INTRODUCTION

Peroxides play at least three distinct roles in the metabolism of arachidonic acid. The most obvious role is as products of the enzymatic oxygenation of 20:4[1] by either PHS or lipoxygenases. A second role is that of a required initiator for those oxygenation reactions (1–3). The work of Lands and colleagues has clearly demonstrated that both PHS (1, 2) and some lipoxygenases (3) require submicromolar levels of hydroperoxide in order to efficiently oxygenate 20:4. The third role of peroxides, in contrast to their role as initiators, is as inactivators of certain enzymes of the 20:4 cascade. PHS (1, 4), prostacyclin synthase (5–10), and 5-lipoxygenase (11) have all been shown to be sensitive to irreversible inactivation by hydroperoxides at concentrations higher than those necessary for activity as initiators.

This duality of roles with regard to enzyme activities has established the importance of hydroperoxide concentration as a qualitative and quantitative effector of 20:4 metabolism. Total oxygenation of 20:4 may be modulated by peroxide effects on PHS or on lipoxygenases, while the distribution of products may be altered via effects on hydroperoxide- and endoperoxide-metabolizing enzymes. Prostacyclin synthase is an endoperoxide-metabolizing enzyme which is particularly sensitive to inactivation by peroxides (5–10).

This inactivation of enzymes by hydroperoxides may be prevented by the presence of compounds which serve as reducing cofactors for peroxidases. Such compounds donate the necessary electrons for the enzymatic reduction of the hydroperoxide to an alcohol, thus decreasing the peroxide level. Many compounds have been shown to act as reducing cofactors for the peroxidase activity of PHS (reviewed in Ref. 12), including the drugs nafazatrom (13, 14)[2] and PB (15–18), and nafazatrom acts via

[1] The abbreviations used are: 20:4, arachidonic acid; PHS, prostaglandin H synthase; PB, phenylbutazone; 15-HPETE, 15-hydroperoxy-5,8,11,13-eicosatetraenoic acid; HHT, 12-hydroxy-5,8,10-heptadecatrienoic acid; PG, prostaglandin; TX, thromboxane; HPLC, high performance liquid chromatography.

[2] 2,4-Dihydro-5-methyl-2-[2-(2-naphthyloxyl)ethyl]-3*H*-pyrazol-3-one.

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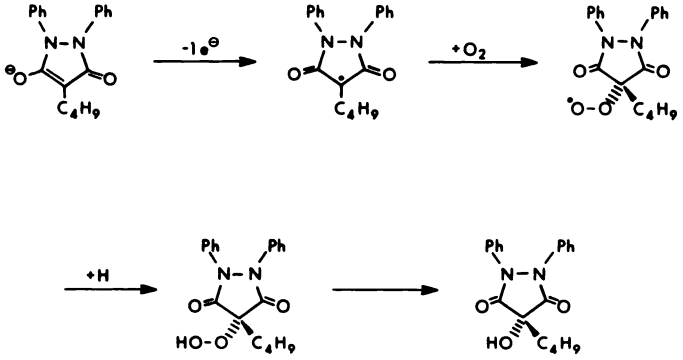
this mechanism to exhibit the expected activity of protecting PHS and prostacyclin synthase from inactivation by hydroperoxides (14). Knowledge of the mechanism of PB oxidation, however, makes such a protective function for PB appear unlikely. PB, like nafazatrom, donates a single electron to the peroxidase during hydroperoxide reduction. The resulting PB radical, however, traps molecular oxygen to yield a peroxyl radical and a PB hydroperoxide[3] enroute to the stable end product 4-hydroxy-PB (Scheme I) (15, 17).



SCHEME 1

The generation of highly reactive oxidant species such as a peroxyl radical and a hydroperoxide, suggests that PB co-oxygenation products may have important effects on 20:4 metabolism. We have investigated the effects of PB co-oxygenation on 20:4 metabolism using ram seminal vesicle microsomes, a preparation containing both PHS and prostacyclin synthase, and report here on the activities and on the mechanism of action of PB in that system.

MATERIALS AND METHODS

Materials. [1-^{14}C]20:4 (56 mCi/mmol) and ^{3}H-labeled standards of 6-keto-$PGF_{1\alpha}$, PGE_2, $PGF_{2\alpha}$, PGA_2, and TXB_2 were supplied by New England Nuclear, Boston, MA. 20:4 (>99%) was a product of NuChek Preps, Inc., Elysian, MN. PB, butylated hydroxyanisole, and soybean lipoxygenase were from Sigma. Nafazatrom was supplied by Bayer AG, West Germany. The lipid hydroperoxide 15-HPETE was prepared by the method of Funk *et al.* (19), and [^{14}C]PGH_2 (0.4 mCi/mmol) was biosynthesized after the method of Hamberg *et al.* (20). [^{14}C]6-Keto-$PGF_{1\alpha}$ (56 mCi/mmol) was prepared and purified via the incubation and HPLC procedures described below. H_2O_2 (30%) and all HPLC solvents were from Fisher. Ram seminal vesicle microsomes were prepared as described previously (21).

Incubation procedure. All studies of 20:4 metabolism were carried out in 0.1 M KH_2PO_4, pH 7.6, in a final volume of 0.5 ml. Incubations contained ram seminal vesicle microsomes and PB or nafazatrom where noted. As microsomal preparations vary widely in their specific activities of both PHS and prostacyclin synthase, PHS activity was measured using a Clark oxygen electrode (Yellow Springs Instruments) immediately prior to incubations, and the amount of microsomal protein used in each subsequent experiment would oxygenate about 90% of the 20:4 added in the control incubations. After a 1-min incubation at 37°, exogenous hydroperoxides were added. Following 3 min of total incubation, [14C] 20:4 in ethanol was added to a final concentration of 20 μM. This incubation procedure ensured that all exogenous peroxide-dependent PB metabolism would be complete prior to the addition of 20:4 (data not shown). Five min after the addition of 20:4, the mixtures were quenched with butylated hydroxyanisole in acetone (50 μl of a 10 mM solution), acidified to pH 3 with dilute HCl, and extracted with 2 × 1.5 ml ethyl acetate. The extracts were evaporated under reduced pressure and the residue was reconstituted in 200 μl of acetonitrile/water/acetic acid (50:50:1) for HPLC analysis. Incubations with [^{14}C]PGH_2 or [^{14}C]6-keto-$PGF_{1\alpha}$ were performed by the procedure described for 20:4 metabolism, except that [^{14}C]PGH_2 was added in acetone to a final concentration of 30 μM in a total volume of 2.0 ml, and [^{14}C]6-keto-$PGF_{1\alpha}$ was added in acetone to a final concentration of 10 μM. Results presented in tables and figures were derived from individual experiments, but are representative of two to four separate experiments.

Analytical procedures. Protein was determined by the method of Lowry *et al.* (22) using bovine serum albumin as the standard. Hydroperoxides were quantitated by iodometric titration (23). PB co-oxygenation was assayed using a Clark oxygen electrode (18). Analysis of labeled products of [^{14}C]20:4, [^{14}C]PGH_2, and [^{14}C]6-keto-$PGF_{1\alpha}$ metabolism was performed by reverse phase HPLC and liquid scintillation counting. HPLC conditions were similar to those previously reported (24). Samples were eluted from a Waters Radial Pak cartridge (C_{18}, 10 μ, 5 mm × 10 cm) at a flow rate of 3.0 ml min^{-1}. Primary prostaglandins were eluted with acetonitrile/water/acetic acid (24:76:1). After 25 min, the solvent composition was changed to 50:50:1 to elute hydroxy acids. Finally, 45 min after injection, the column was washed with acetonitrile for 5 min to elute 20:4. Product identification was inferred from co-chromatography with authentic standards. Thirty-sec fractions were collected throughout each HPLC run and radioactivity was quantitated by liquid scintillation counting. Prostacyclin synthase activity is defined based on the radioactivity co-eluting with 6-keto-$PGF_{1\alpha}$ (*peak 1*, Fig. 2) while PHS activity is based on the sum of 6-keto-$PGF_{1\alpha}$, PGE_2, and HHT (*peaks 1–3*, respectively, Fig. 2).

RESULTS

Initial experiments compared the abilities of PB and nafazatrom to protect PHS and prostacyclin synthase from hydroperoxide-dependent inactivation. The results in Fig. 1 indicate that basal production of prostacyclin, measured as its stable hydrolysis product 6-keto-$PGF_{1\alpha}$, and of total prostaglandins were not significantly affected by either 100 μM PB or 100 μM nafazatrom. When the microsomal preparation was incubated with 15-HPETE prior to 20:4 addition, prostacyclin production and total prostaglandin production were reduced by over 50 and 30%, respectively, relative to the basal production. This effect was abolished by the presence of nafazatrom. PB, however, exhibits no protective function against the

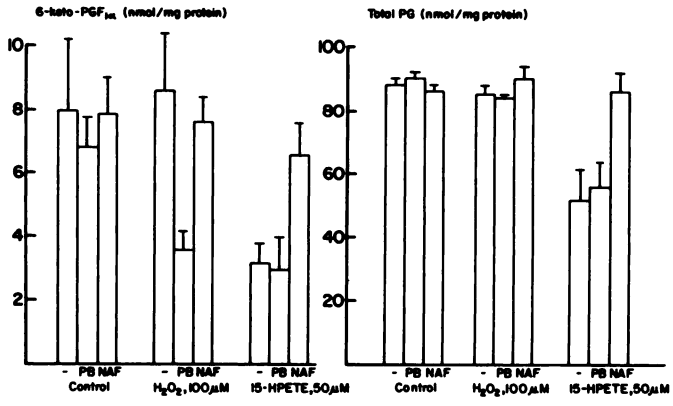


FIG. 1. *Effects of phenylbutazone, nafazatrom, and hydroperoxides on 20:4 metabolism by ram seminal vesicle microsomes*

Ram seminal vesicle microsomes (200 μg of protein/ml) were treated with 100 μM PB, 100 μM nafazatrom, and peroxides, as detailed in Materials and Methods, prior to the addition of 20 μM [^{14}C]20:4. Values represent the mean ± SD of triplicate determinations.

[3] L. J. Marnett, personal communication.

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hydroperoxide-dependent effects on formation of either total prostaglandins or prostacyclin. Even more striking is the result when H_2O_2 is added to the incubations. The H_2O_2 alone had no effect on 20:4 metabolism; however, in the presence of 100 μM PB, a 58% decrease in 6-keto-$PGF_{1\alpha}$ formation was observed. Thus, PB and H_2O_2 produce a synergistic inhibition of the conversion of 20:4 to 6-keto-$PGF_{1\alpha}$.

That this effect on the yield of 6-keto-$PGF_{1\alpha}$ occurs by attenuating the formation of the compound rather than by affecting its consumption is shown by two results. The first is a demonstration of the effect of the PB and H_2O_2 treatment on the resultant HPLC profiles of labeled 20:4 and metabolites (Fig. 2). Under control conditions (Fig. 2A), virtually all of the 20:4 is metabolized, producing predominantly 6-keto-$PGF_{1\alpha}$ (*peak 1*) and PGE_2 (*peak 2*), as well as HHT (*peak 3*). The PB and H_2O_2 treatment (Fig. 2B) depresses conversion of 20:4 to these products, but does not result in the formation of any additional products. In a parallel experiment, labeled 6-keto-$PGF_{1\alpha}$ was added to incubations which had been treated with 100 μM PB, 100 μM H_2O_2, both, or neither agent. Both the recovery and the chromatographic identity of 6-keto-$PGF_{1\alpha}$ are unchanged by any of these treatments (data not shown). This provides a direct demonstration of the stability of 6-keto-$PGF_{1\alpha}$ under our incubation conditions.

As PB undergoes peroxide-dependent oxygenation catalyzed by PHS (15–18), the results in Fig. 1 suggested that this represents metabolic activation of PB *in situ*. This proposition was examined using the antithrombotic drug nafazatrom. Nafazatrom is an efficient competitive inhibitor of PB co-oxygenation (14), and the presence of equimolar nafazatrom and PB totally blocked H_2O_2-dependent PB oxygenation (data not shown). PB and H_2O_2 alone had minimal effects on both the production of 6-keto-$PGF_{1\alpha}$ and on total prostaglandin formation (Table 1). The combination of PB and H_2O_2, however, resulted in a 76% decrease in 6-keto-$PGF_{1\alpha}$ and a 53% decrease in total PG formation. Nafazatrom has no significant effect on 20:4 metabolism in these assays, but is able to block the effects of H_2O_2 and PB on both 6-keto-$PGF_{1\alpha}$ production (50% decrease in the PB and H_2O_2 effect) and total prostaglandin formation (67% decrease).

The PB and H_2O_2-dependent decrease in 6-keto-$PGF_{1\alpha}$ is of particular interest. The heightened sensitivity of 6-keto-$PGF_{1\alpha}$ formation relative to the sensitivity of total prostaglandin formation was demonstrated further by examining the concentration dependence of the PB effect (Fig. 3). In the absence of exogenous peroxide, PB at 100 μM or less had no significant effect on total prostaglandin formation or 6-keto-$PGF_{1\alpha}$ formation in this experiment. In the presence of 100 μM H_2O_2, however, dose-dependent inhibition of both parameters by PB was observed. The

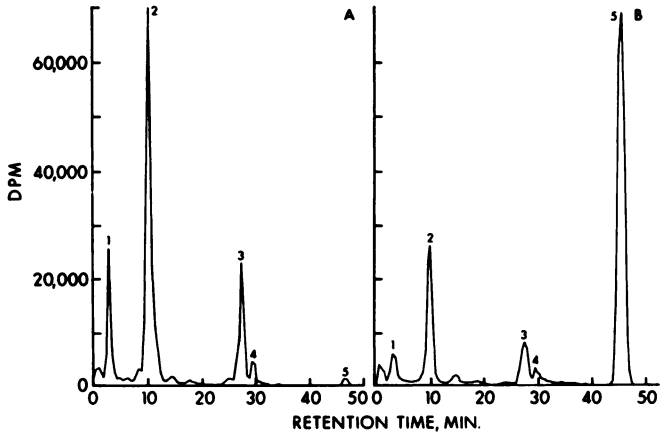

FIG. 2. *HPLC analysis of [^{14}C]20:4 metabolism*

Organic extractable material from incubations was analyzed by reverse phase HPLC (Radial-Pak C_{18}, 10-μ cartridge eluted with an acetonitrile/water/acetic acid step gradient) as detailed in Materials and Methods. Identification of products was inferred from co-chromatography with authentic standards. Panel A, profile of labeled metabolites and 20:4 from an incubation containing 500 μg/ml ram seminal vesicle protein and 20 μM [^{14}C]20:4 in 0.1 M KH_2PO_4, pH 7.6. Panel B, identical to Panel A conditions, except that the microsomes were treated with 100 μM H_2O_2 and 100 μM PB prior to the addition of [^{14}C] 20:4, as described in Materials and Methods. Identities of peaks are: *1*, 6-keto-$PGF_{1\alpha}$; *2*, PGE_2; *3*, HHT; *4*, unknown; *5*, 20:4.

TABLE 1

Effects of phenylbutazone, nafazatrom, and H_2O_2 on 20:4 metabolism

Values represent the mean ± SD of triplicate determinations.

Conditions[a]	H_2O_2	6-Keto-$PGF_{1\alpha}$	Total prostaglandins
	μM	*nmol/mg protein*	
Control	0	21.1 ± 1.5	40.2 ± 0.7
	100	17.4 ± 0.6	38.0 ± 1.0
PB	0	13.6 ± 1.2	35.6 ± 0.4
	100	5.1 ± 0.8	18.8 ± 0.1
Nafazatrom	0	22.0 ± 1.0	39.2 ± 0.2
	100	19.4 ± 0.7	38.8 ± 0.6
PB + nafazatrom	0	19.0 ± 1.5	27.3 ± 3.8
	100	12.9 ± 1.5	33.2 ± 1.3

[a] Results were obtained from incubations containing 400 μg/ml ram seminal vesicle microsomal protein and 20 μM [^{14}C]20:4. PB and nafazatrom, where present, were 100 μM. Details of the incubation and analysis are in Materials and Methods.

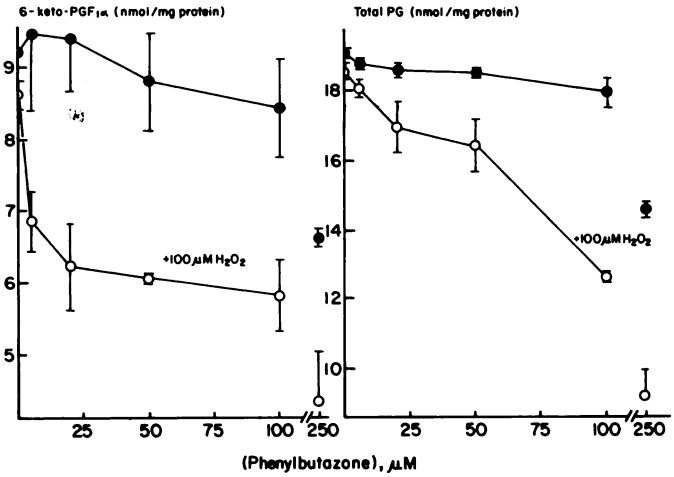

FIG. 3. *Concentration dependence and H_2O_2 dependence of PB effects*

Ram seminal vesicle microsomes (1.0 mg of protein/ml) were treated with the indicated concentrations of PB and H_2O_2 prior to the addition of 20 μM [^{14}C]20:4. Details of the incubation and analysis procedures are in Materials and Methods. All points represent the mean ± SD of triplicate determinations.

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dose dependencies of the inhibition of 6-keto-$PGF_{1\alpha}$ formation and of total prostaglandin formation are radically different. The inhibition of PHS, based on total prostaglandins formed, varies directly relative to the PB concentration throughout the range studied (10–250 μM). The half-maximal effect in this range occurs at about 100 μM PB. Inhibition of prostacyclin biosynthesis appears biphasic, with a half-maximal effect at about 25 μM PB and a more gradual increase in inhibition with PB concentrations above that level.

As the conversion of 20:4 to 6-keto-$PGF_{1\alpha}$ requires the sequential action of PHS and prostacyclin synthase, examination of the effects of PB and H_2O_2 on 20:4 metabolism cannot directly demonstrate inactivation of prostacyclin synthase. Using [^{14}C]PGH_2 as the substrate, however, does provide a direct probe of effects on prostacyclin synthase without superimposition of effects on other enzyme activities. The results of these investigations (Table 2) are similar to those reported using 20:4 as the substrate. Under the basal conditions, approximately 35% of the PGH_2 added was converted to 6-keto-$PGF_{1\alpha}$, with the remainder forming PGE_2 and HHT. The conversion to 6-keto-$PGF_{1\alpha}$ is not affected significantly by H_2O_2 or PB alone, but with both agents present the conversion is inhibited by nearly 40%. As with 20:4, nafazatrom did not affect the basal production of 6-keto-$PGF_{1\alpha}$ but did block the effect of PB and H_2O_2 on that process. The conversion of PGH_2 to 6-keto-$PGF_{1\alpha}$ requires only the activity of prostacyclin synthase; thus, these data provide direct evidence for the inactivation of prostacyclin synthase by PB and H_2O_2.

DISCUSSION

The peroxidative metabolism of PB has been shown to involve the intermediacy of PB peroxyl radical (25) and a hydroperoxide.[3] This peroxidative metabolism of PB occurs not only in the presence of exogenous peroxides, but also during the conversion of endogenously generated PGG_2 to PGH_2 during the normal catalytic cycle of PHS (15, 17). We have examined the effects of these intermediates on two enzymes of 20:4 metabolism. Our data establish that the products of PB oxidation are more potent as inhibitors of prostaglandin biosynthesis than is the parent compound. The extent of the inhibition observed with PB and H_2O_2 varied among different experiments. This is to be expected, in that different microsomal preparations varied in both absolute and relative activities of PHS and prostacyclin synthase. These preparations may also differ in levels of endogenous electron donors and in the peroxide content of microsomal lipid. Any of these variables could alter either the absolute dose-response of the PB effects or the stringency of the requirement for exogenous peroxides. Despite these variations, three observations remained constant throughout our studies. First, exogenous H_2O_2 increased the inhibitory effects of PB on prostaglandin biosynthesis. Second, prostacyclin synthase inactivation was more pronounced than was the effect on PHS. Third, and perhaps most important, the effects of PB and H_2O_2 on 20:4 metabolism could be blocked by the presence of an efficient competing electron donor, nafazatrom, which has been shown to inhibit PB oxidation and to protect enzymes of 20:4 metabolism from inactivation by peroxides (13, 14).

TABLE 2

Effect of phenylbutazone, nafazatrom, and H_2O_2 on PGH_2 metabolism

Mean ± SD of triplicate determinations.

Conditions[a]	H_2O_2	6-keto-$PGF_{1\alpha}$
	μM	*nmol/mg protein*
Control	0	89 ± 13
	100	95 ± 16
PB	0	82 ± 8
	100	56 ± 6
Nafazatrom	0	100 ± 12
	100	102 ± 12
PB + nafazatrom	0	79 ± 9
	100	90 ± 12

[a] 30 μM PGH_2 was added to incubations containing 120 μg/ml ram seminal vesicle microsomal protein as described in Materials and Methods. The concentration of PB and nafazatrom was 100 μM.

The inactivation of prostacyclin synthase by peroxides has been shown to be both concentration-dependent (5–8) and time-dependent (6, 8). These characteristics of the inactivation must be considered in regard to our observation that even at a PB concentration of 250 μM and in the presence of 100 μM H_2O_2 we do not see complete inhibition of prostacyclin biosynthesis. This may indicate that the concentration of activated PB generated is insufficient to totally abolish prostacyclin synthase activity. Alternatively, the 2-min incubation with PB and H_2O_2 prior to the addition of 20:4, while sufficient to allow for all H_2O_2-dependent PB oxygenation to occur, may not allow for the subsequent enzyme inactivation to be complete. It must be emphasized that the peroxidative activation of PB and the subsequent actions of the oxygenated PB derivatives(s) are two separate steps which may proceed at different rates.

The consideration of the time and concentration dependence of enzyme inactivation by peroxides may also be applied to our results using 15-HPETE (Fig. 1). Even though 15-HPETE will trigger PB oxygenation (25), we found no enhancement of the 15-HPETE-dependent enzyme inactivation by PB. The concentration of 15-HPETE which we chose (50 μM) was near the midpoint of the concentration dependence of prostacyclin synthase inactivation in ram seminal vesicle microsomes (14), but is at least 50-fold higher than the peroxide levels proposed as ambient (1, 2). While we did not observe enhancement of 15-HPETE-dependent inactivations by PB at this concentration of lipid hydroperoxide, our data do clearly establish the lack of protective effects of PB against such inactivations. This lack of protection by PB is inconsistent with its role as a peroxidase cofactor, but may be explained by considering the subsequent course of PB metabolism in peroxidative systems (Scheme I).

PB was among a group of compounds examined by Hanel and Lands (26) in a study of the interaction of lipid peroxides and anti-inflammatory drugs. They reach a conclusion opposite to ours in regard to the role of peroxides in the action of PB, i.e., that PB is a more potent inhibitor of PHS in the *absence* of peroxides. This

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discrepancy between their conclusion and ours may be the result of important differences between their experimental design and that used in our work. The concentrations of PB employed in the Hanel and Lands study were from 5- to 40-fold higher than those routinely used in our work. Our data (Fig. 3) suggest that at higher PB concentrations (i.e., 250 μM and above) the enhancing effects of exogenous peroxides may be less important. This may be the case at the 0.5–4.0 mM concentrations of PB used by Hanel and Lands. A second difference between the two studies is in the choice of parameters to be measured. All results in the previous study (26) are based solely on measurement of oxygen consumption. As both 20:4 and PB (15) react with molecular oxygen in this system, measurements of oxygen consumption cannot distinguish between effects on prostaglandin biosynthesis and effects on PB oxygenation. Nevertheless, all effects on oxygen consumption were ascribed to modulation of prostaglandin biosynthesis (26). In contrast, the present work is based on the isolation and quantification of 20:4 metabolites, providing a direct measure of PHS and prostacyclin synthase activities and avoiding the noted uncertainty inherent in the oxygen consumption assay.

Our data do not indicate which of the oxygenated derivatives of PB (i.e., the peroxyl radical or the hydroperoxide) are involved in the inactivation of PHS and prostacyclin synthase. Observations of the inactivation of prostacyclin synthase by hydroperoxides (5–8) suggest that 4-hydroperoxy-PB is involved. The greater sensitivity of prostacyclin synthase relative to PHS towards inactivation by the PB and H_2O_2 treatment also is consistent with the relative sensitivities of these two enzymes to the effects of hydroperoxides. The precedent for hydroperoxide-dependent inactivation of these enzymes is supportive of a role for 4-hydroperoxy-PB as an inactivator in this system, but does not rule out the involvement of the peroxyl radical species as well.

An important feature of the peroxidative activation of PB is found in the stoichiometry of the reaction. Peroxide reduction is a two-electron process, whereas PB oxidation requires the removal of only a single electron. As such, the peroxidative metabolism of PB not only produces a new peroxide, 4-hydroperoxy-PB, but due to the stoichiometry of the oxidation it serves to *increase* the peroxide content of the system rather than to decrease it. This property of PB [and presumably of oxyphenbutazone (27)] is unique among the reducing cofactors for PHS which have been investigated to date.

The behavior of PB in this system raises interesting questions as to the role of PB as an anti-inflammatory drug. Phenidone (16), BW 755C (16), sulindac (28), and acetaminophen (29, 30) and other aminophenols (31) are among the anti-inflammatory drugs which have been shown to be reducing cofactors for peroxidases, particularly PHS. Indeed, a proposed mechanism of action for nonsteroidal anti-inflammatory drugs (reviewed in Ref. 32) postulates that some of these drugs exert their therapeutic action by reducing the levels of hydroperoxides and related oxidants via electron donation to peroxidases. The known intermediates resulting from such peroxidative metabolism of PB (17, 25) and the effects of these intermediates detailed in this work argue against such a mechanism of anti-inflammatory action for PB. If the anti-inflammatory activity of PB results from effects on 20:4 metabolism, then these effects must result from the inactivation of the enzymes responsible for the synthesis of pro-inflammatory eicosanoids rather than by the direct reduction of peroxides. This presents a rather paradoxical case in which PB inhibits the production of lipid mediators of inflammation, but does so via the intermediacy of peroxyl radicals and hydroperoxides which may themselves be pro-inflammatory. Based on the observed toxicity of such reactive oxygen species, this interaction of PB with peroxidases may also represent a step in the mechanism of PB toxicity.

In summary, the interaction of PB with the peroxidase activity of PHS has been shown previously to result in the conversion of PB to a highly reactive peroxyl radical and a hydroperoxide. This interaction serves to amplify the peroxide content of the system and, as we have demonstrated in the present work, yields species which are able to inactivate both PHS and prostacyclin synthase. These effects are currently under investigation to assess the role of peroxidative metabolism of PB in intact cells and tissues.

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