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INHIBITION OF PEROXIDASE ACTIVITY BY SOME NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

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Abstract—The non-steroidal anti-inflammatory drugs, indomethacin, flufenamic acid and naproxen inhibited thyroid peroxidase-catalyzed iodination of BSA *in vitro*. Inhibition by all three drugs was affected more effectively in a hydrogen peroxide generating system than in an incubation system in which hydrogen peroxide was added. Naproxen differed from the other two drugs in so far as it inhibited mainly hydrogen peroxide generation while its comparatively low inhibitory influence (> 20%) on TPO was not dose-dependent. The inhibitory influence of these anti-inflammatory drugs was also observed when other peroxidases, such as lactoperoxidase, chloroperoxidase and horseradish peroxidase were used for catalyzing BSA iodination in a hydrogen peroxide generating system. No iodination of BSA was obtained with horseradish peroxidase when hydrogen peroxide was added instead of generated so that the inhibitory nature of these drugs could not distinguish between their direct effect on horseradish peroxidase or on hydrogen peroxide generation. However, in lactoperoxidase and chloroperoxidase-catalyzed BSA iodinations in non-H_2O_2-generating systems naproxen had no appreciable inhibitory influence below a 1 millimolar concentration. On another thyroid peroxidase activity, namely its catalyzing influence on the exchange reaction between inorganic iodide and organic iodine in diiodotyrosine, indomethacin and naproxen showed unappreciable effects lower than 1 mM concentrations. Similarly, on thyroid peroxidase and lactoperoxidase-catalyzed deiodination of thyroxine they were ineffective inhibitors. The data suggest that these anti-inflammatory drugs are effective inhibitors of iodination reactions but ineffective inhibitors of deiodination reactions.

In iodine metabolism two hydrogen peroxide-dependent peroxidase-catalyzed halogenation reactions are of especial importance. In thyroid hormone formation, thyroid peroxidase (TPO) is involved in different reactions such as iodide oxidation with subsequent iodination of tyrosyl residues [1] and in the coupling of diiodotyrosyl (DIT) residues for thyroxine (T_4) formation [2]. *In vitro* studies also showed that TPO is capable of catalyzing T_4-deiodination [3] and in catalyzing an exchange reaction between iodide an organic iodine in free [3] and in covalently bound DIT-residues [4]. It was also clearly shown that TPO could convert cylindrical TG molecules into ovoid ones in the presence and absence of iodide [5]. Unless these reactions are all interrelated, TPO acts as a multifunctional enzyme. In fact, several other peroxidases of animal (lactoperoxidase and myeloperoxidase) and of plant (chloroperoxidase and horseradish peroxidase) origin can perform similar functions despite structural differences amongst them [6, 7]; for example, a coupling function has been demonstrated for MPO [8] as well as for horseradish peroxidase (HRPO) [9].

Another important peroxidase-catalyzed halogenation reaction concerns the biological defense mechanism of the neutrophil which contains myeloperoxidase (MPO) [7]. A functional difference between TPO and MPO relates to the fact that MPO can iodinate as well as chlorinate; both reactions are effective during phagocytosis as part of the bactericidal action of the inflammatory response although iodination is more effective in antimicrobial systems [10]. Moreover, leucocytes are capable of concentrating iodide [11] and their utilization of thyroid hormones are three to four times greater during phagocytosis over the resting thyroid hormone requirement [12].

Acute infections have been associated with various changes in the thyroid gland and its peripheral hormone levels [13]. On the other hand, the full mechanism of action of non-steroidal anti-inflammatory substances is still moot, except that it is generally agreed that they block prostaglandin synthesis [14, 15] in which a peroxidase enzyme is also responsible for the conversion of PGG_2 to PGH_2 [16]. A classical prostaglandin synthesis inhibitor, such as indomethacin, caused a dramatic decrease in circulating thyroid hormone levels [17] which is generally ascribed to the requirement of prostaglandins for the thyroid secretory response to thyroid stimulating hormone.

In view of the evidence that non-steroidal anti-inflammatory agents may affect three seemingly unassociated phenomena such as thyroid activity, leucocyte activity during the inflammatory response and prostaglandin synthesis and since in all three instances peroxidase enzymes are involved, it became of interest to investigate the influence of anti-inflammatory agents on thyroid peroxidase. Since, however, the peroxidase is H_2O_2-dependent, the effect of some anti-inflammatory agents on a reaction, in which H_2O_2 was added, was compared to one in which H_2O_2 was generated, in order to differentiate between a possible effect on the peroxidase enzyme as compared to an effect on the H_2O_2-generating enzyme.

Because enzymes such as lactoperoxidase (LPO), chloroperoxidase (CPO) and horseradish peroxidase (HRPO) had been shown to be able to catalyze all the reactions of TPO, the influence of non-steroidal anti-

inflammatory agents on the latter enzymes has also been included in the study.

MATERIALS AND METHODS

[^{131}I]- and [^{125}I]Diiodotyrosine (DIT) and thyroxine (T_4). [^{131}I]- and [^{125}I]DIT and T_4 were obtained from the Radiochemical Center, Amersham (40–60 μCi/μg). Both labeled compounds were supplied in 50% propylene glycol. Freshly arrived labeled compounds were used as such. Radioactive materials older than their physical halflives were purified by chromatography on Whatman 3 mm paper in butanol equilibrated with 2 N acetic acid (BAW) by the ascending technique for 16 hr. The radioactive substance was identified and extracted with methanol: 15 N NH_4OH (3:1 v/v), filtered through a millipore filter and concentrated to dryness at 15° in an Evapo-mix (Buchler instruments) and the residue dissolved in 1 mM KOH. In some cases [^{131}I]DIT was prepared from adult rat (200 g) thyroids 24 hr after the injection of 300 μCi ^{131}I per rat. The thyroids were homogenized in 0.1 M-KCl and thyroglobulin was prepared by sucrose density gradients (5–40%) and dialyzed against 0.067 M-PO_4-buffer containing 0.1 M-KCl, pH 7.01, and digested with pronase under reduced pressure. The digest was chromatographed in BAW, extracted with methanol:ammonia and rechromatographed in BAW. Fresh DIT samples prepared in this way were about 90 per cent pure as judged from radioactive counts.

Enzymes. Two preparations of TPO were used. One preparation was a gift from Dr. Alvin Taurog * with a A_{410}/A_{280} ratio of 0.38. The enzyme had been stored at a concentration of 1.95 mg/ml in the frozen state in 0.02 M-phosphate, pH 6.8 for approximately eight years during which time it retained practically all its activity (i.e. ~ 1000 U/mg). The other TPO was prepared in our laboratories from hog thyroids according to Taurog *et al.* [18]. This preparation had approximately one third of the activity of the Taurog preparation as judged by its A_{410}/A_{280} ratio of 0.13 [19].

Lactoperoxidase and horseradish peroxidase were obtained from Miles Laboratories (South Africa) and chloroperoxidase from Sigma Chemical Co. The respective haem/protein absorption ratios ($A_{403-414}/A_{275-280}$) for LPO, HRPO and CPO were 0.74, 1.27 and 0.6. Glucose oxidase was a Hopkins and Williams product.

Incubation system. Two types of incubation systems were used. In the one H_2O_2 was generated by a glucose–glucose oxidase reaction. Incubation time started at the time of addition of glucose oxidase. Incubation was continued for 20 min at 37° and samples were directly applied to the chromatography paper at the end of the incubation period. In the non-H_2O_2-generating system, H_2O_2 was directly added to the medium at 37° at a concentration of 4 μM/ml and the reaction was stopped after one minute by rapid addition of methylmercaptoimidazole (MMI) to a final concentration of 1 mM.

For iodination experiments crystalline bovine serum albumin (BSA) was used at a final concentration of 500 μg/ml. The rest of the components were as follows: TPO (3 μg/ml), ^{131}I-iodide (1 mM), glucose (1 mg/ml), glucose oxidase (0.04 U/ml) and phosphate buffer pH 7.0 (0.067 M). The total volume was 500 μl. For [^{125}I]T_4 delabeling or iodine exchange in [^{131}I]DIT, 50 μM-[^{125}I]T_4 or [^{131}I]DIT was used in the incubation system instead of BSA.

Chromatography and counting of samples as well as stable iodine analyses were performed as described earlier [3].

Anti-inflammatory reagents. 0.05 M stock solutions of indomethacin (m.wt 357.8) naproxen (m.wt 230) and flufenamic acid (m.wt 282) were prepared by dissolving the reagent in 1 M-Tris buffer, pH 11.4. The required concentrations (10^{-7} to 10^{-2}M) were obtained by dilution in 0.067 M-PO_4, pH 7.04. Similar dilutions were made from 1 M-Tris buffer to act as reagent blanks.

RESULTS

A. Peroxidase-catalyzed iodination of BSA. In order to distinguish between a possible effect of the anti-inflammatory reagent on the peroxidase enzyme or on the H_2O_2-generating enzyme, dose–response curves were obtained for both systems as is illustrated in Fig. 1 for TPO. The data indicate that all three anti-inflammatory drugs tested, inhibited iodination of BSA in the H_2O_2-generating system when compared to an identical system to which the drugs were not added. Flufenamic acid and naproxen inhibited iodination virtually to the same extent on an equimolar basis. Naproxen was less effective as an inhibitor at low concentrations (10^{-7} to 10^{-4} M) and at the highest concentration of 10^{-2} M it had only about half of the inhibitory effect than the other two substances.

The inhibitory influence of these drugs on TPO-catalyzed iodination of BSA in a non-H_2O_2-generating system differed from that in the H_2O_2-generating system mainly in two appreciable aspects. First, the inhibitory dose–response curves for both indomethacin and flufenamic acid shifted to the right, indicating a greater inhibitory influence when both enzymes (TPO and GO) were present. Thus an effective inhibitory effect on TPO alone is only exerted at a 1.0 millimolar concentration whereas in the presence of TPO and GO an inhibitory effect is already observed at a 10 micromolar level. Nevertheless, these two drugs inhibit iodination both at the hydrogen peroxide-generating level as well as on TPO directly.

A second obvious difference between the two sets of data lies in the inhibitory nature of naproxen which, at all concentrations, is less effective in the H_2O_2-generating system than the other drugs and seems to be ineffective as an inhibitor of TPO since it only appreciably inhibits GO in the H_2O_2-generating system and showed no dose–response relationship when H_2O_2 was added directly to the incubation system.

The *in vitro* iodination of BSA by LPO in the H_2O_2-generating system was also inhibited by all three anti-inflammatory drugs. In this case, however, the reaction was more sensitive to the inhibitory effect of flufenamic acid than to indomethacin while the naproxen curve (10^{-5} to 10^{-3} M) falls between those of the other two drugs (Fig. 2A). The lower naproxen–inhibition curve in Fig. 2A was obtained in a non-H_2O_2-generating system which again indicated that naproxen had a greater inhibitory effect on LPO plus GO than when

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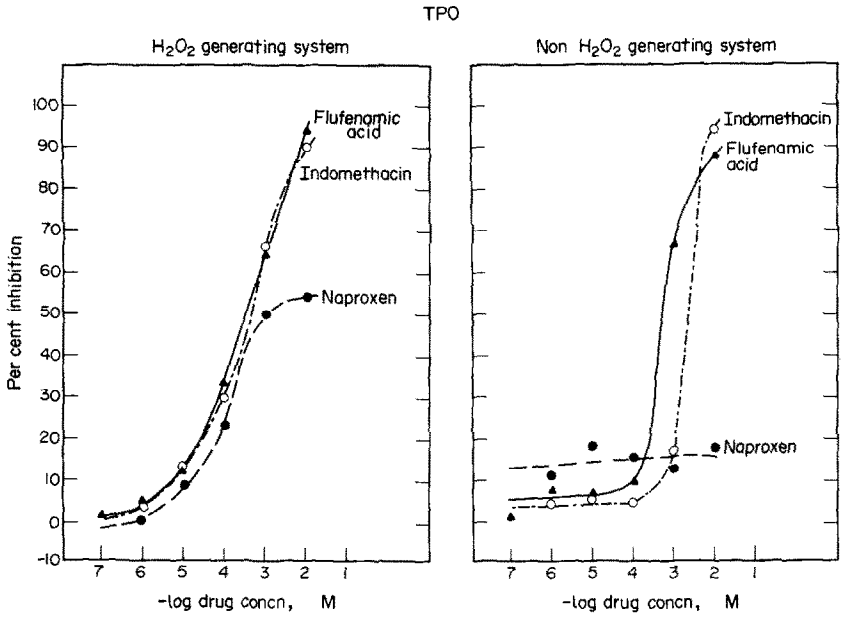

Fig. 1. *In vitro* TPO-catalyzed iodination of BSA as influenced by indomethacin, flufenamic acid and naproxen in a H_2O_2 generating system (left) and a non-H_2O_2 generating system (right). The incubation system for the H_2O_2 generating system contained BSA (500 μg/ml), TPO (3 μg/ml), [^{131}I]iodide (1 mM), glucose (1 mg/ml), glucose oxidase (0.04 U/ml) in 0.067 M-phosphate buffer pH 7.0. After 20 min incubation at 37°, 25 μl was chromatographed for 2 hr in collidine–3 N NH_4OH (100:33 v/v). In the non-H_2O_2-generating system glucose and glucose oxidase were omitted; instead H_2O_2 (4 μM/ml) was directly added at 37° and the reaction stopped after 1 min by rapid addition of methyl mercaptoimidazole to a final concentration of 1 mM.

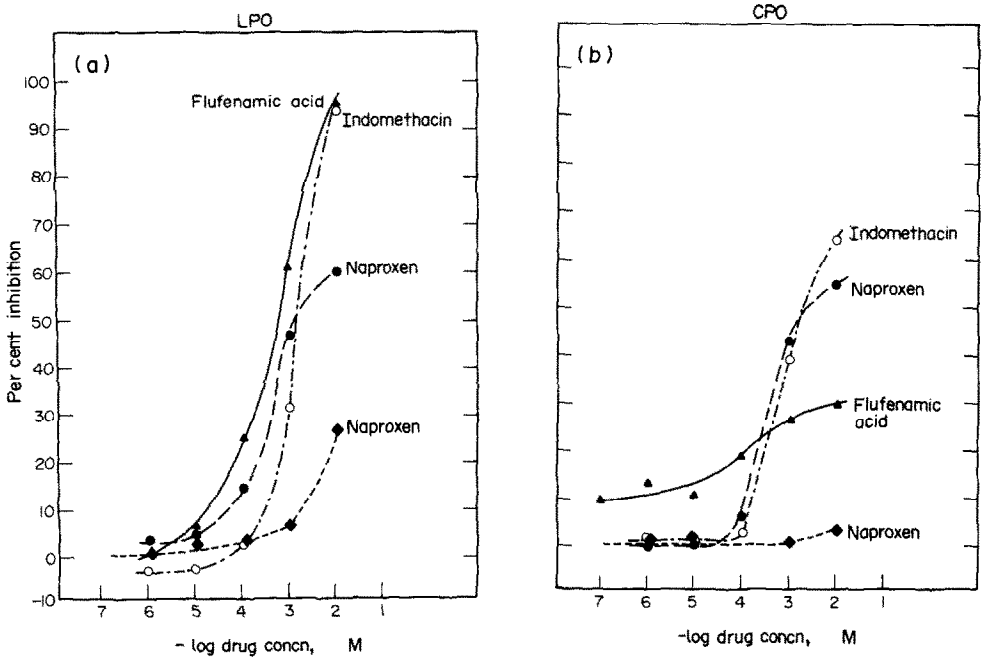

Fig. 2(A) The *in vitro* LPO-catalyzed iodination of BSA as influenced by increased concentrations (10^{-7} to 10^{-2} M) of flufenamic acid, indomethacin and naproxen. The lower naproxen curve was obtained in a system in which H_2O_2 was directly added to the incubation medium whereas the other curves were derived from a H_2O_2-generating system. (B) The *in vitro* CPO-catalyzed BSA-iodination as influenced by flufenamic acid, indomethacin and naproxen. The lower naproxen curve was obtained in a non-H_2O_2 generating system as in (A). Components were as indicated in the legend to Fig. 1 except for the final LPO and CPO concentrations of 18.8 μg/ml each.

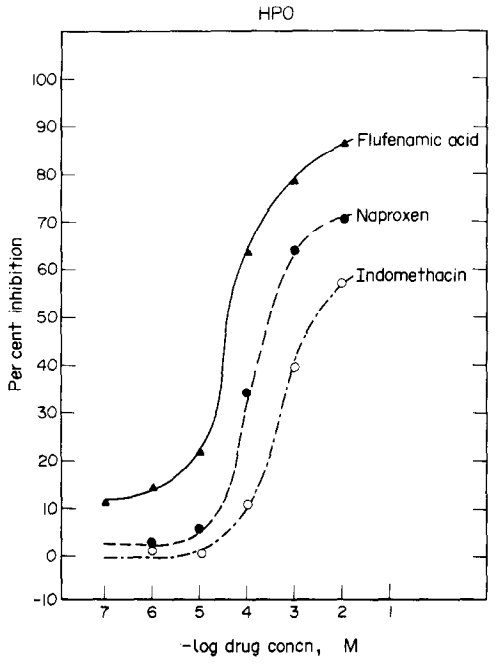

Fig. 3. HPO-catalyzed BSA-iodination as affected by increased concentrations of indomethacin, flufenamic acid and naproxen in a H_2O_2-generating system. Components were as indicated in the legend to Fig. 1 except for a final HPO concentration of 18.9 μg/ml.

LPO was the only enzyme present. It differed however from the TPO-catalyzed BSA iodination (Fig. 1) in so far that naproxen at 10^{-2} M also inhibited LPO-catalyzed BSA iodination in a non-H_2O_2-generating system.

In CPO-catalyzed BSA iodinations (Fig. 2B) the system was less sensitive to the inhibitory nature of the anti-inflammatory drugs. In this case flufenamic acid at lower concentrations tends to show greater inhibitory effects, and at higher concentrations less inhibition of BSA iodination, than indomethacin and naproxen. Again, naproxen showed no inhibitory effect up to a 10^{-2} M concentration.

With HPO-catalyzed BSA iodination in a H_2O_2-generating system flufenamic acid showed a greater inhibitory effect than naproxen whereas indomethacin was the least effective (Fig. 3). When HPO-catalyzed BSA iodination was performed in an iodination system in which H_2O_2 was added instead of generated, no iodination was observed.

B. The influence of indomethacin and naproxen on TPO-catalyzed iodine exchange in DIT. The [^{125}I]DIT was purified by paper chromatography in BAW. When incubated for 20 min in the full incubation medium in the absence of glucose oxidase 86.5 per cent of the activity was present in the DIT-band, in the presence of glucose oxidase only 44.1 per cent, indicating that within the incubation period 51 per cent of the [^{125}I]DIT was delabeled. For comparative purposes these control values were taken as 100 per cent and experimental values calculated accordingly. (Fig. 4).

There was a tendency for the stable iodine in the DIT-band to increase with increased indomethacin concentrations above 1 mM. Part for the reason for this increased difference between control and experimental values may be a greater inhibition of iodine incorporation into DIT than iodine loss due to deiodination of DIT as the indomethacin concentration is increased.

The data on naproxen (Fig. 4B) resemble those of indomethacin, by and large, except that ^{131}I incorporation was inhibited to a larger extent than ^{125}I delabeling at concentrations higher than 100 micromolar. The sudden change after this concentration in iodide exchange was not markedly reflected in the stable iodine change of the DIT-band.

C. The influence of indomethacin, flufenamic acid and naproxen on TPO- and LPO-catalyzed deiodination of T_4. In Table 1 results from different experiments are summarised in which different [^{131}I]T_4 samples had been used. The degree of purity of these samples is

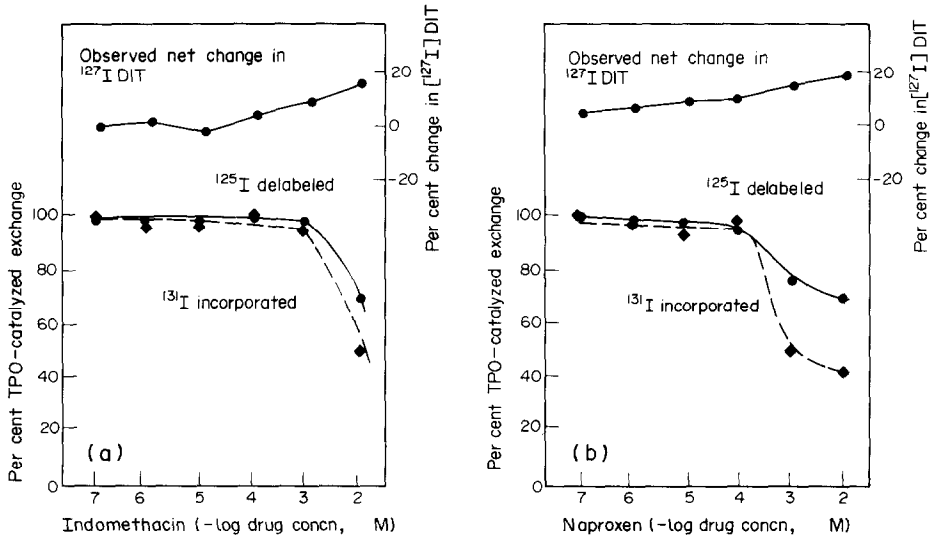

Fig. 4. The influence of indomethacin (A) and naproxen (B) on the TPO-catalyzed exchange reaction between [^{131}I]iodide and [^{125}I]DIT. The reaction mixture contained chromatographically purified [^{125}I]DIT (26 μM). Other components were as indicated in the legend to Fig. 1.

Table 1. The influence of indomethacin, flufenamic acid and naproxen on TPO and LPO-catalyzed deiodination of [^{131}I]T_4 delabeling and ^{125}I incorporation into T_4

Experiment	Peroxidase	Drug (concn)	Per cent ^{131}I in T_4	Per cent ^{131}I in I^-	Per cent ^{125}I incorporated into [^{131}I]T_4
1.	None	None	86.6	3.5	5.8
	TPO*	None	34.2	46.3	6.0
	TPO*	Indomethacin (1 mM)	33.2	44.9	6.0
	TPO*	Naproxen (1 mM)	38.0	46.0	6.0
	TPO*	Flufenamic acid (1 mM)	35.7	43.7	4.3
2.	None	None	82.5	10.7	10.1
	TPO	None	30.7	43.3	5.5
	TPO†	Indomethacin (0.1 μM)	32.9	37.9	6.1
	TPO†	Indomethacin (1.0 μM)	29.7	40.9	5.4
	TPO†	Indomethacin (10 μM)	33.5	39.2	6.6
	TPO†	Indomethacin (100 μM)	32.4	40.5	6.6
	TPO†	Indomethacin (1 mM)	36.5	33.3	6.0
	TPO†	Indomethacin (10 mM)	37.7	30.0	6.3
3.	None	None	67.5	29.6	6.0
	LPO	None	12.9	65.8	6.4
	LPO	Indomethacin (1 mM)	23.0	59.2	6.7
	LPO	Naproxen (1 mM)	15.9	63.4	7.6
	LPO	Flufenamic acid (1 mM)	11.8	57.6	3.3

* Prepared in the South African MRC Iodine Metabolism Research Unit [19].
† Prepared by Dr. Alvin Taurog [18].
Components were as indicated in the legend to Fig. 1. The LPO concentration in the incubation medium was 18.8 μg/ml in experiment 3.

indicative of radiochemical breakdown products which form during storage [20, 21].

In the first experiment summarised in Table 1 a TPO preparation from our laboratory was used which deiodinated [^{131}I]T_4 effectively. Thus the initial ^{131}I in the T_4 chromatographic band decreased from 86.6 to 34.2 per cent after 20 min incubation at 37°. At the same time the ^{131}I activity in the iodide band increased from 3.5 to 46.3 per cent while practically no ^{125}I as iodide was incorporated into T_4. One millimolar concentrations of indomethacin, flufenamic acid and naproxen had no appreciable inhibitory influence on TPO-catalyzed delabeling of T_4.

The influence of indomethacin on TPO-catalyzed delabeling of [^{131}I]T_4 was repeated on a full range of indomethacin concentrations (10^{-7} to 10^{-2} M), using a TPO preparation obtained from Dr. A. Taurog*. The results, portrayed in experiment 2 of Table 1 suggest a slight but ineffective inhibition at the highest TPO concentrations. Once again no ^{125}I incorporation was observed above control values.

When the experiment was repeated with LPO a 2 mM concentration of indomethacin showed approximately a 12 per cent inhibition of LPO-catalyzed delabeling of [^{131}I]T_4 whereas similar concentrations of naproxen and flufenamic acid were ineffective in inhibiting the deiodination reaction. With LPO also no ^{125}I incorporation into [^{131}I]T_4 was observed above those of control values.

DISCUSSION

Thyroid peroxidase can perform a multiple of functions [1–5], while other peroxidases are also effective in catalyzing similar reactions [6–9] although the various peroxidases differ structurally. Nevertheless, functional differences do exist [22], in fact, even for one and the same enzyme, the reaction rate varying with a particular substrate [23]. However, the mechanism whereby any one reaction is catalyzed by a particular peroxidase may be similar, whereas the process whereby a particular function will be inhibited may differ. No attempt was made to test the influence of anti-inflammatory agents on any particular peroxidase reaction at its optimum pH, yet care was exercised to compare the influence of the drug with a control experiment in which the reagents were identical except for the absence of the anti-inflammatory agent in the control experiment. This means that different drug concentrations contained different Tris concentrations since Tris was used to bring the anti-inflammatory drugs in solution from which they were diluted with a phosphate buffer. Experiments performed on different Tris concentrations used in the dilutions showed that Tris had no inhibitory influence on peroxidase-catalyzed iodination of BSA. Moreover, the Tris content for any particular drug concentration was matched in its control.

Our results on BSA iodination agree with those of previous workers on the iodination of TG [24] which indicated that HRPO was not a very effective catalyst and that the enzyme is much more sensitive to H_2O_2 than the other enzymes [7]. Thus no iodination could be obtained in a non-H_2O_2-generating system with this enzyme and it was therefore not possible to compare the mechanism of action of the anti-inflammatory agents on HRPO between the two systems. Nevertheless, in all other peroxidases it is quite clear that naproxen acted differently from indomethacin and flufenamic acid in so far as it had little or no influence on the peroxidase enzymes, but exerted its influence mainly on H_2O_2 generation. The superoxide radical had been demonstrated as an intermediate in the mechanism of action of

galactose oxidation for H_2O_2 formation [25]. It could therefore also be expected to form during H_2O_2 generation by glucose-glucose oxidase. It therefore seems possible that the anti-inflammatory agents inhibit iodination reactions in one of three ways i.e. by inhibiting the electron transport chain whereby less O_2^- and less H_2O_2 will form [26] or by absorbing or destroying free radicals [28, 29]*, or by competing with the tyrosyl residue or iodine for peroxidase and hydrogen peroxide or peroxidase–hydrogen peroxide complexes as had been suggested for the way in which indomethacin inhibited the oxidation of *O*-dianisidine [27].

On the basis of our data it seems unlikely that the *in vivo* efficiency of different non-steroidal anti-inflammatory agents can be compared with their *in vitro* inhibitory influence on the peroxidase-catalyzed oxidation of a substrate as suggested by Saeed and Warren [27] for HRPO oxidation of *O*-dianisidine. The efficiency of these drugs in inhibiting peroxidase-catalyzed BSA iodinations in a H_2O_2-generating system varies from one enzyme to the other. Thus for TPO, there is little difference in the effective inhibition of BSA-iodination between flufenamic acid and indomethacin at any concentration, while naproxen is less effective at all concentrations. In the HRPO data indomethacin is much less effective than either flufenamic acid or naproxen whereas in the CPO experiments flufenamic acid at lower concentrations is more effective and at high concentrations less effective than either indomethacin or naproxen. The only consistent findings were that they are all inhibitory to a greater or lesser extent and that naproxen is either ineffective or very much less effective as an inhibitor in a H_2O_2-non-generating system that in a H_2O_2-generating system in TPO and CPO-catalyzed iodinations.

The mechanism whereby the peroxidases catalyze T_4-deiodination is unclear [19]. It is obvious, however, that the anti-inflammatory reagents tested were far less effective inhibitors of the peroxidase-catalyzed deiodination reactions or of reactions in which deiodination forms a part, such as in the iodine exchange reaction in DIT, than they were inhibitors of BSA iodination. Furthermore, not all peroxidase-catalyzed reactions were influenced by any one anti-inflammatory agent to the same extent. In experiments which we performed on sonificated leucocytes containing myeloperoxidase, we confirmed previous observations of a stimulating effect of the antithyroid substance, methylmercaptoimidazole (MMI) on MPO-catalyzed delabeling of $[^{125}I]T_4$ whereas it inhibited TPO-catalyzed deiodination of T_4. MMI stimulated MPO-catalyzed delabeling of $[^{125}I]T_4$ between 20 to 62 per cent above control levels between 10^{-7} to 10^{-3} M concentrations of MMI whereas the same MMI concentrations affected a drop in MPO-catalyzed BSA iodination of sonificated leucocytes from 100 per cent to zero in a H_2O_2-generating system (unpublished results). In this case the iodination function was profoundly inhibited whereas the deiodination reaction was grossly stimulated by the same substance.

Rosenberg *et al.* [30] concluded from their studies that several peroxidases in an *in vitro* system, containing H_2O_2 or glucose–glucose oxidase, could affect a loss of iodine from iodine labeled amino acids including T_4 but that the loss of label was attributable to iodine exchange reactions and was not due to deiodination. Our results [3] indicated, however, that TPO caused deiodination as observed by a net loss of stable T_4 during the reaction. Results reported here (Table 1) again show that neither TPO or LPO catalyzed incorporation of ^{125}I into ^{131}I-labelled T_4 above those of control values in which the peroxidases were omitted from the incubation medium in a H_2O_2 generating system. It is clear therefore that peroxidase enzymes catalyze iodine exchange in DIT but not in T_4, in which only deiodination is catalyzed, and that these reactions are inhibited by antithyroid substances such as MMI, except in MPO-catalyzed deiodination of T_4 which is stimulated by MMI. Data presented here and experiments we performed on sonificated leucocyte preparations indicate that the anti-inflammatory drugs tested are all inhibitors of peroxidase catalyzed iodination of BSA but are poor inhibitors of peroxidase-catalyzed iodine exchange in DIT and of T_4-deiodination.

Prostaglandins mimic a number of effects produced by thyrotropin (TSH), such as adenylate cyclase stimulation, glucose oxidation, iodide trapping and organification, colloid droplet formation and hormone secretion [31]. Although aspirin, which is the classical non-steroidal anti-inflammatory drug and well-known prostaglandin synthesis inhibitor [14], was not included in the present study, it seems feasible that the inhibitory effects of aspirin and of indomethacin on TSH-dependent iodide organification in rat thyroids [31] and on thyroid hormone secretion in the dog [32] may be related to their possible inhibitory effects on TPO or on H_2O_2-generation.

The *in vitro* effects of the non-steroid anti-inflammatory agents do not permit the extrapolation that these drugs will inhibit peroxidase-catalyzed iodinations *in vivo* at therapeutic concentrations. Nevertheless, if half of the permitted therapeutic dose were absorbed it can be calculated that much higher blood levels will be obtained on a single dose than that which caused an effective inhibition of TPO-catalyzed BSA iodination *in vitro*. It is therefore possible that long-term treatment may result in a decrease in circulating thyroid hormone levels [17] which, together with increased leucocyte utilization of thyroid hormones during the inflammatory response [12], may cause concern.

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* Work quoted by Saeed and Warren in [27].

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