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Effect of Nonsteroidal Antiinflammatory Drugs on Plasma Protein-Thyroxine Interaction (38133)

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A number of studies have shown that all the nonsteroidal antiinflammatory drugs can be bound to serum albumin either at the same sites or at closely located sites (1) and can displace certain substances, including tryptophan (2, 3), sulfonamide (4, 5), uric acid (6), pyridoxal phosphate (7), thiopental (8) and warfarin (9). The studies on this line have recently proposed the tentative hypothesis that nonsteroidal antiinflammatory drugs manifest their effects by displacing peptides (10) or pyridoxal phosphate (7) from their binding sites on the proteins.

Christensen first showed that salicylate displaced thyroxine from the binding sites of plasma protein *in vitro* (11). Since that time, a number of studies had indicated that salicylate did actually displace thyroxine from the binding sites of plasma protein and made more thyroxine available to the cells in man (12–14) and experimental animals (15–19). It is not known, however, whether other nonsteroidal antiinflammatory drugs similarly displace thyroxine from the binding sites of plasma protein or whether thyroxine displacing activity varies greatly from drug to drug. Because of physiological importance of free thyroxine (20, 21), *in vitro* and *in vivo* experiments were performed to study the relative activity of 9 nonsteroidal antiinflammatory drugs in displacing thyroxine from the binding sites of plasma proteins.

Materials and Methods. One hundred and thirty five male Wistar rats, weighing 180–300 g, were used in experiments 1–3. The compounds used as nonsteroidal antiinflammatory drugs were sulpyrine, ibufenac, acetyl salicylate, sodium salicylate, bucolome, oxyphenbutazone, indomethacin, phneylbutazone, flufenamic acid and mefenamic acid.[1] In experiment 1, the compounds were dissolved in Krebs–Ringer phosphate buffer (KRP) (pH 7.4) to obtain appropriate concentrations. KRP was also used to dilute rat plasma (15 x) and labeled thyroxine.[2] One hundred sixty-four pieces of 50 mg diaphragms were obtained from 41 animals. Labeled thyroxine solution (0.2 ml), diluted rat plasma (0.2 ml) and the solution of test materials (0.2 ml) were mixed, and the diaphragm was added. The uptake of labeled thyroxine by muscle was measured as reported previously (22). In experiment 2, 5 ml of blood was obtained from each of 30 animals by cardiac puncture, using heparinized syringes. The plasma was separated and kept in a deep freezer until use. Two ml of plasma was mixed in a triiodothyronine resin uptake kit with a 0.1 ml of a tracer dose of labeled thyroxine and 0.1 ml of test materal, and the whole system was incubated in a water bath at 24° for 60 min. The washing of the resin sponge and the calculation of thyroxine uptake were performed according to the methods given in the direction accompanying the kit. In experiment 3, beginning 14 days after surgical thyroidec-

[1] The test substances were obtained from following co. Sulpyrin, Acetyl Salicylate, Sodium Salicylate, Bucolome (Takeda Chemical Industries Co. LTD.), Oxyphenbutazone, Phenylbutazone (Fujisawa Pharmaceutical Co.LTD.), Flufenamic acid, Mefenamic acid (Sankyo Co.LTD.), Indomethacin (Sumitomo Pharmaceutical Co.LTD.), Ibufenac (Kaken Pharmaceutical Co.LTD.).

[2] Labeled thyroxine (^{131}I-thyroxine, specific activity 35–42 mCi/mg) was obtained from Abbott Laboratories.

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TABLE I. Effect on Nonsteroidal Antiinflammatory Drugs on Muscle Uptake of Labeled Thyroxine *in vitro*.

Exp Drug	Concentration of the drug M	No. of trials	Muscle uptake of labeled thyroxine %	% of control	P value
1. Control	KRP buffer	4	15.3 ± 0.6^a	100	
Sulpyrine	$3 \times 10^{-9} M$	4	16.2 ± 1.0	106	N.S.
	$3 \times 10^{-8} M$	4	17.8 ± 1.2	116	N.S.
	$3 \times 10^{-7} M$	4	18.6 ± 0.6	121	< 0.025
2. Control	KRP buffer	4	15.5 ± 0.8	100	
Acetyl salicylate	$3 \times 10^{-9} M$	4	14.3 ± 1.8	92	N.S.
	$3 \times 10^{-8} M$	4	18.8 ± 1.0	121	N.S.
	$3 \times 10^{-7} M$	4	26.0 ± 1.4	168	< 0.005
3. Control	KRP buffer	4	16.5 ± 0.9	100	
Sodium salicylate	$3 \times 10^{-9} M$	4	17.4 ± 1.0	105	N.S.
	$3 \times 10^{-8} M$	4	21.1 ± 1.8	128	N.S.
	$3 \times 10^{-7} M$	4	28.7 ± 1.0	174	< 0.005
4. Control	KRP buffer	4	14.8 ± 0.8	100	
Bucolome	$7.5 \times 10^{-9} M$	4	16.0 ± 0.6	108	N.S.
	$1.5 \times 10^{-8} M$	4	16.3 ± 0.5	110	N.S.
	$3 \times 10^{-8} M$	4	18.5 ± 1.0	125	< 0.05
	$6 \times 10^{-8} M$	4	22.3 ± 1.0	151	< 0.005
5. Control	KRP buffer	4	17.4 ± 0.6	100	
Oxyphenbutazone	$9 \times 10^{-10} M$	4	18.0 ± 0.8	103	N.S.
	$4.5 \times 10^{-9} M$	4	22.0 ± 0.8	126	< 0.01
	$2.3 \times 10^{-8} M$	4	23.4 ± 1.2	186	< 0.01
	$4.5 \times 10^{-8} M$	4	35.4 ± 1.4	203	< 0.005
6. Control	KRP buffer	4	15.0 ± 1.2	100	
Indomethacin	$3 \times 10^{-9} M$	4	20.2 ± 1.4	135	< 0.05
	$6 \times 10^{-9} M$	4	20.4 ± 0.8	136	< 0.01
	$3 \times 10^{-8} M$	4	30.6 ± 0.8	204	< 0.005
	$6 \times 10^{-8} M$	4	30.8 ± 1.2	205	< 0.005
7. Control	KRP buffer	4	14.2 ± 0.8	100	
Phenylbutazone	$3 \times 10^{-9} M$	4	20.6 ± 0.4	145	< 0.005
	$1.5 \times 10^{-8} M$	4	27.6 ± 0.6	194	< 0.005
	$3 \times 10^{-8} M$	4	30.6 ± 0.8	215	< 0.005
	$7.5 \times 10^{-8} M$	4	31.0 ± 1.0	218	< 0.005
8. Control	KRP buffer	4	15.2 ± 0.8	100	
Flufenamic acid	$1.1 \times 10^{-10} M$	4	18.0 ± 0.8	118	< 0.01
	$5.5 \times 10^{-10} M$	4	22.0 ± 1.4	145	< 0.01
	$2.8 \times 10^{-9} M$	4	28.2 ± 1.2	187	< 0.005
	$5.5 \times 10^{-9} M$	4	29.2 ± 0.6	192	< 0.005
9. Control	KRP buffer	4	15.7 ± 1.0	100	
Mefenamic acid	$1.2 \times 10^{-10} M$	4	27.5 ± 1.0	175	< 0.005
	$6 \times 10^{-10} M$	4	34.8 ± 1.5	222	< 0.005
	$1.2 \times 10^{-9} M$	4	39.9 ± 1.8	254	< 0.005

[a] mean $\pm$ SE. P value indicates the significance when compared with the control.

TABLE II. Effect of Mefenamic Acid and Flufenamic Acid on Resin Uptake of Labeled Thyroxine in the Presence of Plasma.

Group	No. of trials	% of uptake of labeled thyroxine by resin
Control	5	10.2 ± 0.3 [a]
Mefenamic acid 2×10^{-4} M	5	14.8 ± 0.4
Flufenamic acid 2×10^{-4} M	5	12.9 ± 0.3

[a] Mean ± SE. Control-Mefenamic acid $P < 0.005$. Control-Flufenamic acid $P < 0.005$. Mefenamic acid-Flufenamic acid $P < 0.01$.

tomy, 64 rats were fed a low iodine diet[3] and were injected with a solution of labeled thyroxine (0.5 μCi) + stable thyroxine (2 μg) and 1 ml of test material (1.2×10^{-2} M) daily intraperitoneally for 10 days. Feces were collected for the last 2 days and fecal radioactivity was expressed as % of daily administered dose. Twenty-four hours after the last injections, blood was obtained by cardiac puncture and plasma PBI was measured by the isotopic technique (23).

Results. Experiment 1. Effect of nonsteroidal antiinflammatory drugs on in vitro uptake of labeled thyroxine by muscle in the presence of plasma

By measuring the muscle uptake of labeled thyroxine in the presence of plasma, a possible effect of 9 antiinflammatory compounds on plasma protein-thyroxine interaction was assessed *in vitro*. As shown in Table I, some variations of the control values were found from experiment to experiment, but the magnitude of variation was rather small so as to make possible the approximate comparison of the 9 drugs. All 9 antiinflammatory compounds augmented the muscle uptake of labeled thyroxine, but the magnitude of the increase varied greatly from compound to compound. For instance, the increase was significant only when high concentrations (3×10^{-7} M–3×10^{-8} M) of sulpyrine, acetyl salicylate and bucolome were used. In sharp contrast, the increase was significant at the concentration of 4.5×10^{-10} or 1.2×10^{-10} M when flufenamic acid and mefenamic acid were used. The increase was intermediate when oxyphenbutazone, indomethacin and phenylbutazone were tested.

Experiment 2. Effect of antiinflammatory compounds on resin uptake of thyroxine in the presence of plasma

The resin uptake of labeled thyroxine was 10.2% when no test material was added to the plasma (Table II). This uptake increased significantly when mefenamic acid was added (final concentration 2×10^{-4} M). Also, the resin uptake of labeled thyroxine increased significantly after addition of flufenamic acid (final concentration 2×10^{-4} M). However, the increase of resin uptake by mefenamic acid was significantly more than that by flufenamic acid.

Experiment 3. Effect of antiinflammatory compounds on plasma PBI and fecal excretion of thyroxine

The *in vivo* effect of antiinflammatory drugs on plasma protein-thyroxine interaction was studied by measuring plasma PBI and fecal loss of labeled compound in thyroidectomized rats treated with stable and labeled thyroxine. As shown in Fig. 1 A, plasma PBI was 3.6 μg/100 ml and approximately 43% of the radioactivity administered daily as labeled thyroxine was excreted into feces in the control animals. When bucolome, oxyphenbutazone and flufenamic acid were administered, plasma PBI decreased significantly. Flufenamic acid had the greatest effect, bucolome the least and oxyphenbutazone intermediate. In contrast, fecal radioactivity markedly increased in response to the administration of 3 antiinflammatory drugs. Quite interestingly, a decrease of plasma PBI inversely correlated with an increase of fecal radioactivity.

A similar type of experiment was performed using ibufenac, sodium salicylate and phenylbutazone. As shown in Fig. 1 B, plasma

[3] Composition of low iodine diet was reported previously (Endocrinology 79:138, 1966).

PBI decreased in response to the administration of antiinflammatory drugs. Phenylbutazone had the greatest effect, sodium salicylate intermediate and ibufenac the least. In contrast, fecal radioactivity increased after administration of these 3 drugs. Again, a decrease of plasma PBI inversely correlated with an increase of fecal radioactivity.

Discussion. Since it has been assumed generally that the availability of thyroxine to the cells depends upon the relative binding capacity of the plasma proteins and perhaps the specific binding protein for thyroxine in the cells (20, 21), we have studied the effect of salicylate on plasma protein-thyroxine interaction by measuring muscle uptake of labeled thyroxine *in vitro*. As previously reported (24) and confirmed here, sodium salicylate apparently interfered with the plasma protein-thyroxine interaction *in vitro* as evidenced by a marked increase of muscle uptake of labeled thyroxine. Furthermore, it was found that this thyroxine displacing activity is not specific to salicylate but common to 8 other nonsteroidal antiinflammatory drugs. Since free thyroxine is the active component available to the cells *in vivo* for TSH regulation, degradation, excretion and initiation of hormonal action (25), and since a number of antiinflammatory drugs have been used clinically, the assessment of relative potencies of these drugs in increasing free thyroxine is of theoretical and practical importance. Quite interestingly, mefenamic acid and flufenamic acid have the strongest, oxyphenbutazone, indomethacin and phenylbutazone have the intermediate and sulpyrine, acetyl salicylate, sodium salicylate and bucolome have the least effect on plasma protein-thyroxine interaction *in vitro*. Thus the relative potencies of thyroxine displacing activity of these compounds are roughly comparable to those of urate (6), thiopental (8) and pyridoxal phosphate displacing activities. Furthermore, this order of potency is roughly comparable to the relative potency of the drugs in stabilizing serum albumin (26, 27), in blocking the aggregation of red blood cells (28, 29) and in inhibiting release of lactic dehydrogenase and acid phosphatase from platelet (30). Further studies are required to elucidate whether or not thyroxine displacement is in some way involved in the manifestation of the action of nonsteroidal antiinflammatory drugs.

In vitro thyroxine displacing activity of the compounds may not necessarily indicate *in vivo* thyroxine displacing activity, since *in vivo* disappearance of the drugs may be unequal. Thus an *in vivo* experiment is in order to assess this thyroxine displacing action of the antiinflammatory drugs in terms of physio-

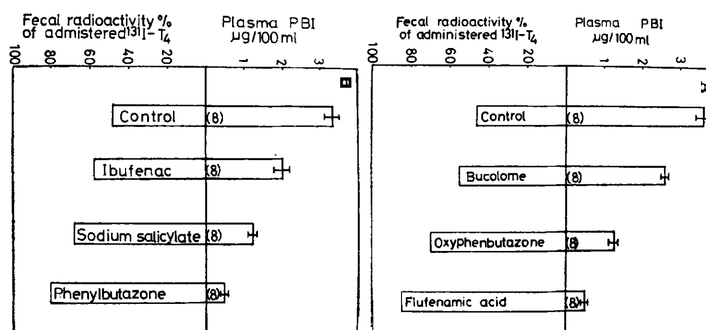

FIG. 1. Effect of nonsteroidal antiinflammatory drugs on plasma PBI and fecal loss of labeled thyroxine. Beginning 14 days after thyroidectomy, animals were injected with solutions of labeled thyroxine (0.5 μCi) + stable thyroxine (2 μg) and test material daily intraperitoneally for 10 days. Twenty four hour after the last injections, blood was obtained and plasma PBI was calculated by isotopic technique. Feces were collected and fecal radioactivity was expressed as percent of daily administered dose. Bars and vertical lines indicated mean ± SE. Parenthesis indicated the number of animals used. Statistical analysis for PBI:

A; Control-Bucolome $P < 0.005$
Bucolome-Oxyphenbutazone $P < 0.005$
Oxyphenbutazone-Flufenamic acid $P < 0.1$

B; Control-Ibufenac $P < 0.005$
Ibufenac-Sodium Salicylate $P < 0.005$
Sodium Salicylate-Phenylbutazone $P < 0.005$

logical importance. Our present study clearly indicated that 6 nonsteroidal antiinflammatory drugs depressed plasma PBI in thyroidectomized, thyroxine-treated animals. Furthermore, this decrease was apparently due to an increased clearance of free thyroxine by the liver, since a decrease of plasma PBI was inversely related with an increase of fecal loss of thyroxine. On the basis of our previous studies (23, 31–34), it can be concluded that nonsteroidal antiinflammatory drugs do displace thyroxine from the binding sites of plasma protein *in vivo* despite a great variance of their chemical formula. No apparent discrepancy was found between *in vitro* and *in vivo* thyroxine displacing activity of the drugs.

Summary. Effect of nonsteroidal antiinflammatory drugs on plasma protein-thyroxine interaction was studied by measuring muscle or resin uptake of labeled thyroxine *in vitro* or by measuring plasma PBI and fecal loss of thyroxine in thyroidectomized rats treated with stable and labeled thyroxine. All 9 nonsteroidal antiinflammatory drugs tested displaced thyroxine from the binding sites of plasma protein *in vitro*. Mefenamic acid and flufenamic acid had the strongest, oxyphenbutazone, indomethacin and phenylbutazone had the intermediate and sulpyrine, acetyl salicylate, sodium salicylate and bucolome had the least effect. Six nonsteroidal antiinflammatory drugs depressed plasma PBI and increased fecal loss of thyroxine by displacing thyroxine from the binding sites of plasma protein *in vivo*. The magnitude of *in vivo* thyroxine displacing activity correlated well with that of *in vitro* displacing activity.

1. Paulus, H. E., and Whitehouse, M. W., Ann. Rev. Pharmacol. **13**, 107 (1973).

2. McArthur, J. N., Dawkins, P. D., Smith, M. J. H. and Hamilton, E. B. D., J. Pharm. Pharmacol. **23**, 32 (1971).

3. Smith, H. G. and Lakatos, C., J. Pharm. Pharmacol. **23**, 180 (1971).

4. McQueen, E. G., Brit. J. Pharmacol. **36**, 29 (1969).

5. McQueen, E. G. and Wardell, W. M., Brit. J. Pharmacol. **43**, 312 (1971).

6. Bluestone, R., Kippen, I., Klinenberg, J. R. and Whitehouse, M. M., J. Lab. Clin. Med. **76**, 85 (1970).

7. Skidmore, I. F. and Whitehouse, M. W., Biochem. Pharmacol. **15**, 1965 (1966).

8. Chaplin, M. D., Roszkowski, A. P. and Richards, R. K., Proc. Soc. Exp. Biol. Med. **143**, 667 (1973).

9. Aggeler, P. M., O'Reilly, R. A., Leong, L. and Kowitz, P. E., New Engl. J. Med. **276**, 496 (1967).

10. McArthur, J. N., Dawkins, P. D., Smith, M. J. H. and Hamilton, E. B. D., Brit. Med. J. **1**, 677 (1971.)

11. Christense, L. K., Nature **183**, 1189 (1959).

12. Austen, F. K., Rubini, M. E., Meroney, W. H. and Wolff, J., J. Clin. Invest. **37**, 1131 (1958).

13. Hetzel, B. S., Good, B. F., Wellby, M. L. and Begg, M. W., Aust. Ann. Med. **11**, 34 (1962).

14. Woeber, K. A. and Ingbar, S. H., J. Clin. Invest. **43**, 931 (1964).

15. Good, B. F., Potter, H. A. and Hetzel, B. S., Austr. J. Exp. Biol. Med. **43**, 291 (1965).

16. Good, B. F., Hetzel, B. S. and Opit, L. J., J. Endocr. **21**, 231 (1960).

17. Osorio, C. and Myant, N. B., Endocrinology **72**, 253 (1963).

18. Good, B. F., Hetzel, B. S. and Hogg, B. M., Endocrinology **77**, 674 (1965).

19. Flock, E. V. and Owen, C. A., Jr., Endocrinology **77**, 475 (1965).

20. Robbins, J. and Rall, J. E., Recent Prog. Horm. Res. **13**, 161 (1957).

21. Ingbar, S. H. and Freinkel, N., Recent Prog. Horm. Res. **16**, 353 (1960).

22. Yamada, T., Whallon, J., Tomizawa, T., Shimoda, S. and Shichijo, K., Metabolism **14**, 281 (1965).

23. Yamada, T., Endocrinology **81**, 1285 (1967).

24. Takemura, Y., Yamada, T., Ozawa, K. and Shichijo, K., Metabolism **15**, 679 (1966).

25. Yamada, T., Tomizawa, T., Takemura, Y., Katagai, S., Kajihara, A., Aoki, K., Kobayashi, I., Onaya, T., Yagi, S. and Shichijo, K., Gunma Symp. Endocrinol. **1**, 307 (1964).

26. Mizushima, Y., Sakai, S. and Yamaura, M., Biochem. Pharmacol. **19**, 227 (1970).

27. Grant, N. H., Alburn, H. E. and Kryzanauskas, C., Biochem. Pharmacol. **19**, 715 (1970).

28. Görög, P. and Kovacs, I. B., J. Pharm. Pharmacol. **22**, 86 (1970).

29. Brown, J. H., Taylor, J. L. and Waters, I. W., Proc. Soc. Exp. Biol. Med. **136**, 137 (1971).

30. Glenn, E. M. and Bowman, B. J., Proc. Soc. Exp. Biol. Med. **130**, 1327 (1969).

31. Yamada, T. and Jones, A. E., Endocrinology **82**, 47 (1968).

32. Takemura, T., Yamada, T. and Shichijo, K., Metabolism **15**, 566 (1966).

33. Kobayashi, I., Yamada, T. and Schichijo, K., Metabolism **18**, 800 (1969).

34. Fukuda, H., Yamada, T. and Shichijo, K., Arch. Intl. Pharmacodyn. **182**, 161 (1969).

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