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Research Article

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Intrinsic Mineralocorticoid Agonist Activity of Some Nonsteroidal Anti-Inflammatory Drugs

A POSTULATED MECHANISM FOR SODIUM RETENTION

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ABSTRACT Because some nonsteroidal anti-inflammatory drugs (NSAID) induce salt and water retention and exhibit other steroid-like actions, studies were performed to ascertain whether these drugs possess intrinsic mineralocorticoid agonist activity. In vitro competitive binding assays utilizing tissue from adrenalectomized rats demonstrated that some NSAID can displace [³H]-aldosterone from renal cytoplasmic mineralocorticoid receptors. Displacement potency for these sites was in the sequence: aldosterone > spironolactone > phenylbutazone (PBZ) > aspirin (ASA) > indomethacin (IDM). Concentration ratios required to obtain significant displacement of [³H]aldosterone were high but clearly within the therapeutic range for PBZ and ASA but not IDM. The analogues oxyphenbutazone (OBZ) and sodium salicylate (SS) were similar in binding activity to PBZ and ASA, respectively. Lineweaver-Burk analysis revealed that the inhibition of [³H]aldosterone binding was competitive in nature. In addition, PBZ was shown to prevent the nuclear binding of [³H]aldosterone. In vivo injection of PBZ and ASA resulted in competition for [³H]aldosterone renal binding comparable to the in vitro studies. Administration of PBZ and OBZ to adrenalectomized rats resulted in significant salt retention whereas ASA and SS did not differ significantly from controls. Salt retention elicited by PBZ and OBZ was inhibited by spironolactone, a competitive mineralocorticoid antagonist. These data suggest that, despite nonsteroidal structures, PBZ and OBZ induce salt retention via a receptor-mediated mineralocorticoid pathway analogous to aldosterone action.

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INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAID)¹ such as aspirin (ASA), phenylbutazone (PBZ), and indomethacin (IDM) are commonly used agents whose mechanism of action is incompletely understood (1). Recent work has suggested that the inhibition of prostaglandin synthesis plays a role in the anti-inflammatory action of these drugs (2). However, many therapeutic effects as well as side effects of NSAID resemble responses evoked by adrenal steroids (1, 3). A large body of evidence indicates that NSAID do not act via the pituitary-adrenal axis (1, 3-6). Although these drugs are structurally unrelated to the adrenal steroids, the available data are not inconsistent with the hypothesis that NSAID are themselves weak steroid agonists. Of interest in this respect are the findings that various NSAID, in addition to being anti-inflammatory, have been shown to elicit the following steroid-like effects: (a) retention of sodium with resultant increases in plasma volume (7-12), (b) suppression of lymphocyte transformation (13), (c) antagonism of the antiminerocorticoid effects of spironolactone (11, 14, 15), (d) occasional improvement in the condition of patients with Addison's disease (16), and (e) suppression of the pituitary-adrenal axis (17, 18).

In the light of these observations, it seemed appropriate to reevaluate whether NSAID could be corticosteroid agonists. Since it is currently felt that all steroid hormones initiate their action by binding to specific cytoplasmic receptors in target tissue (19), we considered

¹Abbreviations used in this paper: NSAID, nonsteroidal anti-inflammatory drugs; ASA, aspirin; PBZ, phenylbutazone; IDM, indomethacin; OBZ, oxyphenbutazone; SS, sodium salicylate.

it pertinent to ascertain whether any NSAID could compete for mineralocorticoid receptor binding sites. If these compounds possess significant binding affinity for aldosterone receptors, in addition to possessing salt-retaining activity, a case could be made for intrinsic mineralocorticoid agonist properties. Further, if the mineralocorticoid effects could be blocked by competitors of receptors binding, additional supportive evidence for this hypothesis would be provided.

METHODS

[1, 2-³H]Aldosterone, 52 and 54 Ci/mmol, was purchased from New England Nuclear (Boston, Mass.). The following drugs were generously donated by the respective companies: IDM and dexamethasone by Merck Sharp & Dohme (West Point, Pa.), spironolactone by G. D. Searle & Co. (Chicago, Ill.), and PBZ and oxyphenbutazone (OBZ) by CIBA Pharmaceutical Co. (Summit, N. J.). Unlabeled A-grade aldosterone was purchased from Calbiochem (San Diego, Calif.), acetyl salicylic acid (ASA) was from Sigma Chemical Co. (St. Louis, Mo.) and sodium salicylate (SS) was from Fisher Scientific Co. (Pittsburgh, Pa.). The drugs were used without further purification. Unlabeled steroids were dissolved in absolute ethanol and further diluted with buffered saline. Final alcohol concentration was always less than 1%. Radioactive steroids were diluted in saline, and the solvents were evaporated under a nitrogen stream. SS was directly dissolved in buffer. For some *in vitro* studies, PBZ, OBZ, ASA, and IDM were dissolved in alcohol and used as described for the steroids. For *in vivo* studies and for the higher *in vitro* concentrations, PBZ, OBZ, and ASA were dissolved in 0.2 N NaOH, titrated back to pH 7.4 with dilute HCl, and brought to volume with Tris buffer. Solutions of drugs were made fresh daily.

Preparation of tissue. Male Sprague-Dawley rats of 100–200 g body weight were adrenalectomized at least 3 days before use. For *in vitro* studies, the rats were stunned by a blow to the head and rapidly decapitated, and the viscera were perfused via the vena cava with 50 ml of phosphate-buffered iced saline to wash out blood. The kidneys were removed, decapsulated, halved, sliced at 275 μ m with an automatic tissue chopper (McIlwain), (Brinkman Instruments, Inc., Westbury, N. Y.), and rinsed under suction.

***In vitro* mineralocorticoid receptor binding studies.** Mineralocorticoid receptor binding was studied in kidney slices incubated with 5.2 nM [³H]aldosterone. 10-fold excess dexamethasone was added to all flasks to prevent aldosterone binding to glucocorticoid sites (20, 21). Competing drugs were added simultaneously at the indicated concentrations, and the incubations were performed in Eagle's minimal medium with Hanks' balanced salt solution (Grand Island Biological Co., Grand Island, N. Y.) in a shaking water bath at 25°C for 40 min. At the conclusion of the incubation, the slices (~250 mg/flask) were rinsed on nylon mesh under suction and homogenized in 2.5 ml of 0.25 M sucrose, 3 mM EDTA, 10 mM Tris-HCl, pH 7.4 with a motor-driven Teflon-glass homogenizer. This and all subsequent procedures were performed at 0°–4°C. The homogenate was made 20% with respect to glycerol and centrifuged at 100,000 g for 60 min to obtain a cytosol fraction. Protein-bound [³H]aldosterone was assayed by filtration of cytosol through G-50 Sephadex columns as previously described (20, 21). Aliquots of the void volume were taken for radioassay in Aquasol (New England Nuclear) and

for protein determination by spectroscopy at 260:280 nm (22). In all binding studies, "nonspecific" binding (high capacity, low affinity) was defined as that binding of ³H-steroid resistant to 1,000-fold excess of the same unlabeled steroid. This was determined by parallel studies in all experiments and was subtracted from the total binding to give "specific" binding.

Mineralocorticoid receptor depletion studies were performed in the same manner except that adrenalectomized rats were injected *i.p.* with aldosterone or PBZ 20–30 min before sacrifice. Control rats received only vehicle solution. Residual binding was then assayed as described above, and the number of mineralocorticoid sites depleted by a given treatment was calculated from the difference between binding in slices from control and treated rats.

In those experiments in which nuclear binding was assessed, similar conditions were again employed except that weighed kidney slices were incubated for 1 h. The slices were then homogenized in 0.25 M sucrose, 3 mM CaCl₂, 10 mM Tris-HCl, pH 7.4. Subsequently the homogenate was centrifuged at 800 g for 10 min and the pellet was then washed three times. The wash consisted of resuspending the nuclear pellet vigorously with a Vortex mixer (Scientific Industries, Inc., Queens Village, N. Y.) in iced homogenization buffer; each wash was followed by centrifugation at 800 g for 10 min to yield the washed nuclear fraction. Nuclear-bound [³H]aldosterone was then extracted with 0.3 M KCl. After centrifugation, an aliquot of extract was taken for radioassay of [³H]aldosterone. Binding resistant to 1,000-fold excess aldosterone was subtracted from all values.

***In vivo* binding studies.** Adrenalectomized rats were anesthetized with 30 mg/100 g chloral hydrate given *i.p.* The femoral vein was surgically exposed, and injections of various competing drugs or vehicle were made *i.v.* These were immediately followed by a second injection from a separate syringe of [³H]aldosterone and 10-fold dexamethasone. The concentration of ³H-steroid injected was 0.35 nmol/100 g, and the competing drugs were given at 100,000-fold molar excess. Between 4 and 5 min after injections, a blood sample was obtained by cardiac puncture for subsequent determination of NSAID plasma concentration. The kidneys were then removed, sliced, and rinsed, and bound [³H]aldosterone was determined in the cytosol fraction by Sephadex chromatography as described above. Plasma salicylates were measured by the ferric chloride method (23), and PBZ and OBZ were generously assayed by Dr. G. Lukas of CIBA-GEIGY Corp. (Summit, N. J.).

Electrolyte excretion studies. Adrenalectomized rats previously maintained on 0.9% saline and Purina Rat Chow (Ralston Purina Co., St. Louis, Mo.) were placed in individual metabolic cages at 8 a.m. No food was provided, and the saline drinking water was fortified with 5% glucose. Urine was collected for 2–3 h (control period), and the animals were then injected with various drug combinations *i.p.* Dosages were calculated per 100 g body weight according to the following schedule: aldosterone, 1 μ g; spironolactone, 2 mg; SS and ASA, 10 mg; PBZ and OBZ, 7.5 mg. The latter three drugs were dissolved in 0.2 N NaOH, titrated back to pH 7.4 with HCl, and brought to 1 ml with Tris buffer. Control rats received 1 ml of vehicle solution. The urine output over the subsequent 2 h was collected and the experimental period urine was then collected over the ensuing 3 h, (i.e., between 2 and 5 h after drug administration). Urinary sodium and potassium concentrations were determined on an Instrumentation Laboratory, Inc. (Lexington, Mass.) flame photometer, and creatinine was assayed by a modification of the Taussky method (24).

The rats appeared to tolerate these doses of NSAID without difficulty and were reused on an alternating schedule over several weeks.

RESULTS

In vitro mineralocorticoid binding studies. NSAID, particularly PBZ and its analogues, exhibit salt and water retention as prominent side effects (7, 8). To determine whether this NSAID effect could be mediated by direct interaction with the mineralocorticoid receptors, competition studies were performed for [³H]aldosterone binding sites in kidney cytosol. These experiments were performed with kidney slices in the presence of 10-fold excess unlabeled dexamethasone to block any [³H]-aldosterone binding to glucocorticoid receptors in the kidney (20, 21). As shown in Fig. 1, unlabeled aldosterone predictably displaced the bound [³H]aldosterone. Spironolactone, a clinically useful mineralocorticoid antagonist, displayed significant potency in these competition studies as previously reported (25). PBZ and OBZ demonstrated approximately equivalent potency, both requiring a concentration of about 100,000-fold excess to inhibit 50% of the [³H]aldosterone binding. ASA was slightly more potent than SS, however neither achieved 50% inhibition at a 500,000-fold excess. IDM did not significantly displace any [³H]aldosterone at a concentration ratio of 10,000:1 whereas at 100,000:1, there was displacement of 21% of the bound [³H]-

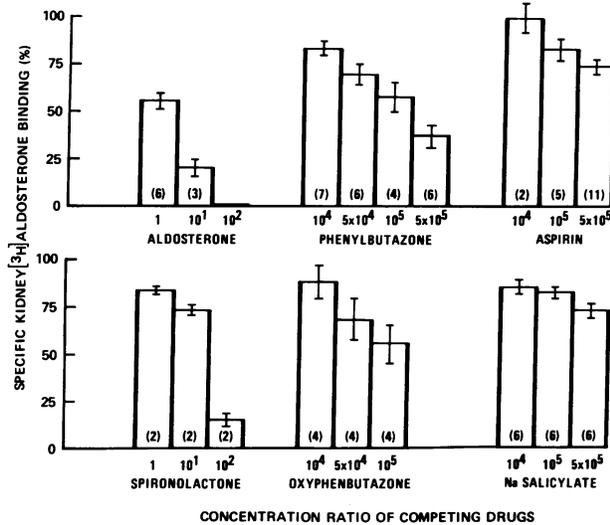


FIGURE 1 Competition studies for [³H]aldosterone binding sites in kidney. Kidney slices were incubated with 5.2 nM [³H]aldosterone plus 10-fold dexamethasone ± competing drugs for 40 min at 25°C in a shaking water bath. Bound steroid in the cytosol was determined by Sephadex chromatography. Total specific [³H]aldosterone binding in the absence of competitor was 16 fmol/mg cytosol protein. The results shown are the means ± SE, the figure within the bar indicates the number of experiments.

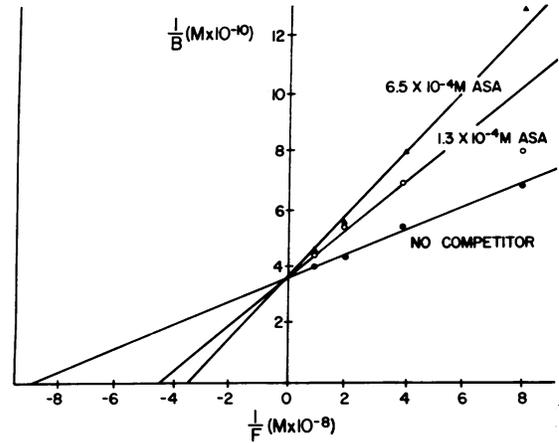


FIGURE 2 Lineweaver-Burk analysis of ASA inhibition of [³H]aldosterone binding. Kidney slices were incubated with varying concentrations of [³H]aldosterone (1.3–10 nM) plus 10-fold dexamethasone in the presence or absence of the indicated concentrations of ASA. After 40 min incubation at 25°C the bound steroid was determined in the cytosol fraction by Sephadex chromatography. Each point represents the mean specific binding of three determinations.

aldosterone (data not shown). Since this concentration ratio exceeds the therapeutic ratio attained when IDM is used clinically, the significance of the demonstrated competition is unclear. Because of its failure to compete at lower concentration ratios, IDM was not utilized in subsequent experiments.

The nature of the inhibition of cytoplasmic binding of [³H]aldosterone by ASA and PBZ was further explored at various concentrations of radioactive steroid. The data are shown in Figs. 2 and 3 plotted by the method of Lineweaver and Burk. The apparent $K_{i, 25^\circ\text{C}}$ for aldosterone is ~ 1 nM in agreement with previous studies (20). In the presence of inhibitor, the plotted lines intersect at a common point on the ordinate indicating that the inhibition of binding is competitive in nature.

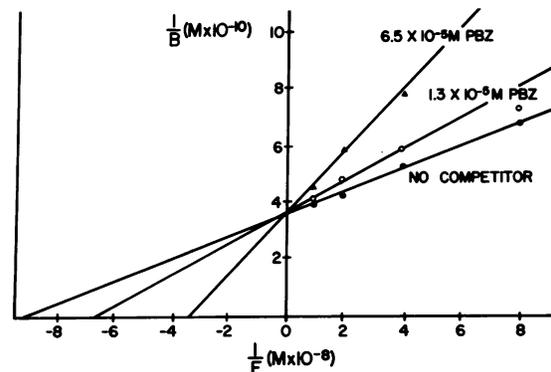


FIGURE 3 Lineweaver-Burk analysis of PBZ inhibition of [³H]aldosterone binding. Same conditions as Fig. 2.

TABLE I
In Vitro Competition for Renal Mineralocorticoid Nuclear-Binding Sites

	No.	Concentration	Percent of control binding
Aldosterone	9	5.2 nM	67 ± 3
PBZ	14	0.52 mM	49 ± 5

Kidney slices from adrenalectomized rats were incubated with 5.2 nM [³H]aldosterone plus 10-fold dexamethasone ± competitors for 60 min at 25°C. After homogenization a crude nuclear fraction was prepared and washed by centrifugation and resuspension three times. Bound [³H]aldosterone was extracted from the washed pellet with 0.3 M KCl. The nuclear binding in the presence of 1,000-fold unlabeled aldosterone was subtracted from all values. Control [³H]aldosterone binding was 0.3 pmol/g kidney slices. The results are the mean ± SE of the indicated number of experiments.

Additional binding studies were performed to insure that competition by PBZ for cytoplasmic-binding sites also blocked the nuclear binding of [³H]aldosterone. At a concentration ratio of 100,000:1, PBZ inhibited ~50% of the [³H]aldosterone nuclear binding, whereas unlabeled aldosterone at a concentration equivalent to [³H]aldosterone, inhibited 33% of the binding sites (Table I).

In vivo competitive binding studies. The ability of NSAID to inhibit cytoplasmic binding of [³H]aldosterone in kidney was also tested in vivo. Kidney was obtained 5 min after the i.v. injection of drugs to anesthetized rats. The doses of NSAID injected were 35

TABLE II
In Vivo Competition by NSAID for Renal Mineralocorticoid Receptors

	Percent of control binding	Plasma concentration
		mg/100 ml
PBZ	(5) 52 ± 6	(4) 35
OBZ	(5) 69 ± 6	(5) 23
ASA	(6) 65 ± 9	(5) 16
SS	(4) 70 ± 9	—

NSAID were injected i.v. at a dose of 35 μmol/100 g. Rats were then injected with [³H]aldosterone plus 10-fold dexamethasone at a dose of 0.35 nmol/100 g. 5 min after the injection, plasma was obtained to determine NSAID concentrations and the kidneys were removed. ³H-Steroid binding was determined in the kidney cytosol by Sephadex chromatography. The total specific [³H]aldosterone binding in the absence of any competitor was 0.2 fmol/mg cytosol protein. The results presented are means ± SE, and the figures in parentheses indicate the number of experiments.

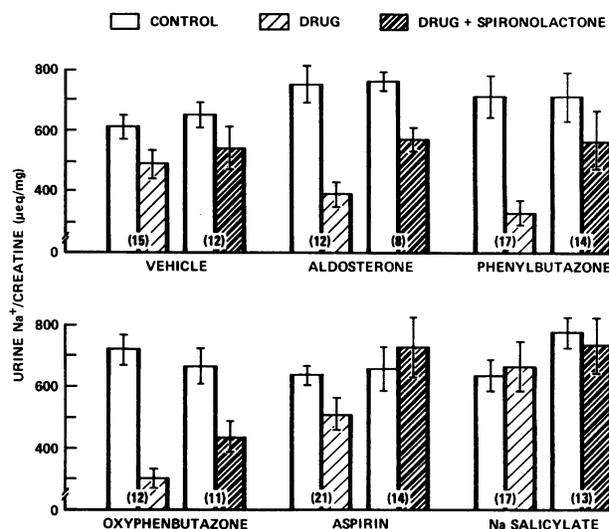


FIGURE 4 The effect of NSAID on urinary sodium excretion and the inhibitory action of spironolactone. Adrenalectomized rats were maintained on 0.9% saline drinking water plus 5% glucose. Control urines were collected for 2–3 h, and the rats were injected i.p. according to the following dosage regimen per 100 g body weight: aldosterone, 1 μg; PBZ and OBZ, 7.5 mg; ASA and SS, 10 mg; and one group of animals received an equivalent volume of vehicle. The experimental urine was collected between 2 and 5 h after injection. Inhibition studies were performed in parallel except that spironolactone 2 mg/100 g body weight was added to the dosage regimen. Results are the mean ± SE of Na⁺ microequivalents per milliliter per creatinine milligram per milliliter. The figures within the bars indicate the number of experiments.

μmol/100 g (~10 mg/100 g for PBZ and OBZ and ~6 mg/100 g for ASA and SS). This was immediately followed by a separate injection of [³H]aldosterone and 10-fold dexamethasone at a molar ratio of 1:100,000 (i.e., 0.35 nmol/100 g). Parallel experiments utilizing 1,000-fold excess of the appropriate unlabeled steroid instead of NSAID obliterated most of the binding. The binding resistant to this treatment was considered “non-specific.” As shown in Table II, [³H]aldosterone binding was significantly diminished by prior NSAID injection. Inhibition of [³H]aldosterone binding by individual drugs was in the same range as achieved in the in vitro studies (Fig. 1). Plasma concentration of NSAID 5 min after an i.v. bolus was 16 mg/100 ml for ASA, 23 mg/100 ml for OBZ, and 35 mg/100 ml for PBZ (Table II).

Mineralocorticoid physiological effects. Mineralocorticoid physiological effects were studied by measuring changes in urinary electrolytes after injection of NSAID or aldosterone into adrenalectomized rats. Additional studies were performed in the presence of spironolactone antagonism to further document that any changes in electrolyte excretion were mediated via mineralo-

corticoid receptors. As can be seen in Fig. 4, aldosterone at 1 $\mu\text{g}/100\text{ g}$ resulted in significant Na^+ retention. Spironolactone at 2 mg/100 g did not alter the Na^+ excretion but, when administered with aldosterone, inhibited most (about 85%) of the mineralocorticoid effect. PBZ and OBZ at 7.5 mg/100 g exhibited significant mineralocorticoid potency which was approximately equivalent to that elicited by aldosterone. Spironolactone also inhibited this mineralocorticoid effect. ASA and SS demonstrated minimal if any mineralocorticoid potency when compared to the changes seen in control animals.

Receptor depletion studies. The effect on Na^+ retention elicited by a 7.5 mg/100 g dose of PBZ appeared roughly equivalent to the effect of a 1 $\mu\text{g}/100\text{ g}$ dose of aldosterone (Fig. 4). Yet this concentration ratio of PBZ (10,000:1 molar ratio) achieved only $\sim 20\%$ competition in the *in vitro* slice system (Fig. 1). In an attempt to determine the actual occupancy rate of mineralocorticoid receptors attained by this dose of PBZ *in vivo*, the following study was performed. Aldosterone or PBZ was injected into adrenalectomized rats at the same dosage and under the same conditions as for the physiological studies. 20–30 min later the kidneys of these rats were removed and residual [^3H]aldosterone cytoplasmic-binding sites measured (Table III). Compared to control animals receiving vehicle, aldosterone depleted 80% and PBZ depleted 43% of the total mineralocorticoid receptors.

DISCUSSION

The results of *in vitro* and *in vivo* competitive binding studies indicate that some of the NSAID tested diminish [^3H]aldosterone binding to mineralocorticoid receptor sites. The inhibition of binding appears to be competitive by Lineweaver-Burk analysis. In addition, this effect can be reproduced *in vivo*. Whereas PBZ, OBZ, ASA, and SS achieved significant displacement of [^3H]aldosterone at therapeutic concentrations, IDM failed to do so, suggesting that this property is not present in all NSAID. It was further demonstrated that PBZ also inhibits nuclear binding of [^3H]aldosterone. Thus, there are several lines of evidence indicating that these binding results are not merely nonspecific drug-protein phenomena but rather specific, dose-dependent and competitive interactions of certain NSAID with mineralocorticoid receptors. Although competitive binding assays are an indirect approach, it appears likely from these experiments that some of the NSAID are binding to the receptor thus inhibiting the attachment of [^3H]aldosterone. However, assuming that an NSAID can bind to the receptor, these experiments fail to distinguish between binding of the ligand to the same site as aldosterone or an allosteric site. More importantly, it must be

TABLE III
Comparative Depletion of Renal Mineralocorticoid Receptors after In Vivo Administration of Aldosterone or PBZ

	Dosage	Percent of control binding
	<i>/100 g</i>	
Aldosterone	1 μg	20 \pm 5.4
PBZ	7.5 mg	57 \pm 8.7

Adrenalectomized rats were injected *i.p.* with either PBZ or aldosterone at the indicated dosage. Control rats received vehicle. 20–30 min later the kidneys were removed and slices incubated with 5.2 nM [^3H]aldosterone plus 10-fold dexamethasone and the number of residual cytoplasmic-binding sites determined. Control rats bound 0.3 fmol/mg cytosol protein. The results are the means \pm SE for three experiments.

elucidated whether the resultant complexes are functionally active as mineralocorticoids. To answer these questions we turned to physiological studies attempting to evaluate the mineralocorticoid potency of those NSAID possessing binding activity.

Of the drugs tested, PBZ and OBZ were the most potent inhibitors of [^3H]aldosterone binding. In the physiological studies these drugs definitively demonstrated sodium-retaining activity. That this activity is receptor mediated is supported by the inhibition of the salt retention by spironolactone, a competitive inhibitor of mineralocorticoid-receptor binding (25, 26). However, the relevance of these findings to the clinical setting remains an open issue. Consideration must be given to species variation and other factors to determine the role of this postulated mechanism in humans. Variation in the rate of metabolism of PBZ exists between species, the half-life in the rat being 3–6 h and in man about 72 h (27). Thus the effects seen in the rat would probably be magnified in humans because of the much slower clearance rate.

Since the binding events being studied are competitive, the dosages selected have emphasized molar ratios of NSAID to physiological levels of steroids. Assuming aldosterone concentrations of 10 ng/100 ml and therapeutic concentrations of ASA $\sim 20\text{ mg}/100\text{ ml}$ (3), PBZ $\sim 10\text{ mg}/100\text{ ml}$ (7, 27), and IDM $\sim 0.1\text{ mg}/100\text{ ml}$ (28, 29), then the molar ratio to aldosterone is in the range of $\sim 10^7$ for ASA, $\sim 10^6$ for PBZ, and $\sim 10^4$ for IDM. While the demonstrated binding activity of ASA and PBZ easily fall within these ratios, IDM fails to achieve significant displacement of [^3H]aldosterone at this dose range. Although the concentration ratio method is a simplistic device that does not take into account species differences, extensive binding of NSAID to plasma proteins, clearance rates, and other differ-

ential pharmacological factors, it provides some estimate of the significance of the concentrations used.

The results obtained in the *in vivo* studies supplement the *in vitro* results. Also, concentrations of drugs measured within 5 min of *i.v.* administration were within the same order of magnitude as the usually accepted concentrations achieved by oral administration measured 24 h later (3, 7, 27). Additionally, the results in the physiological experiments add further support to the hypothesis. The dosages utilized were not excessive, and the rats showed no ill effects despite the fact that they were more susceptible to stress because of their adrenalectomized status.

It is noteworthy that PBZ and OBZ exhibited roughly equivalent potency to aldosterone when administered at a molar ratio of 10,000:1 in the physiological experiments, whereas in the binding studies, a 100,000:1 ratio is required for equivalent competitive potency. In an attempt to explain this discrepancy, the depletion studies were performed employing the same doses used in the physiological experiments. *In vivo*, PBZ and aldosterone both appeared to achieve higher receptor occupancy rates (Table III) than in *in vitro* competition (Fig. 1), but aldosterone remained relatively more potent at the time point examined. Although the explanation for the difference mentioned is not clear from the data available, it should be noted that PBZ has a significantly longer half-life in the rat than aldosterone, possibly allowing for a more prolonged effect *in vivo*. The relationship between binding affinity and functional potency is complex involving multiple variables including absorption, plasma binding, metabolic clearance rate, and other factors.

ASA and SS, although demonstrating some competitive potency in the binding studies, failed to elicit sodium retention. Previous workers have differed in their conclusions as to whether salt retention follows ASA administration (9–11, 30). The absence of a clear-cut biological response in the presence of binding suggests that the drug-receptor complexes formed are either suboptimally active or inactive (19, 26, 31). In the absence of agonist potency, one would predict that the salicylates would function as competitive aldosterone antagonists. This possibility was not tested. ASA is not usually associated with alterations in salt metabolism as a side effect, however several reports in man and experimental animals have indicated that modest doses of ASA inhibit the diuretic action of spironolactone (11, 14, 15). Because of the low affinity demonstrated for the mineralocorticoid receptors by ASA, it is unlikely that inhibition of spironolactone diuresis is due to mineralocorticoid enhancement by an additional weak agonist. Rather, it seems that other mechanisms are involved,

possibly the induction by ASA of an increase in the metabolic clearance rate of spironolactone.

It is well known that PBZ and OBZ commonly cause salt and water retention. It would seem that the mechanism described here is the basis for this side effect. It is of interest that both PBZ and OBZ are active in the binding system and the physiological system, suggesting that both drugs possess activity. This conclusion is supported by the *in vivo* studies where assays for PBZ indicated that the parent compound was still present in the blood at a time that significant competition had occurred. A metabolic product common to both drugs may be the active principal but, in accord with the binding data, it would have to be formed *in vitro*. IDM is also known to result in salt and water retention (3) but to a lesser extent. The binding studies with IDM are negative in the therapeutic range. However, some competition for [³H]aldosterone binding sites was noted at higher concentrations. The possibility exists that this drug may function as a mineralocorticoid in humans, but this would require the invocation of an additional mechanism to overcome the discrepancy in dosage such as species variation or metabolism to a more potent compound. Further study on this point appears warranted.

Although we failed to detect significant glucocorticoid-binding activity or enzyme induction in preliminary experiments, we are not convinced that NSAID do not, in part, function as glucocorticoid agonists as well. Recent studies have demonstrated the ability of several NSAID to induce hepatic tyrosine aminotransferase, a glucocorticoid inducible enzyme, even in adrenalectomized rats (32). Perhaps the greatest difficulty with this hypothesis is reconciling the structural differences between NSAID and steroids. Yet if we accept the thesis that some NSAID have intrinsic mineralocorticoid agonist activity, we must be cautious in eliminating a similar mechanism for glucocorticoid activity. Further studies on this point are presently being carried out.

The significance of the structural aspects of these results should be emphasized. We are postulating that nonsteroidal compounds (*i.e.* PBZ and OBZ) can achieve significant binding in what are considered to be highly specific steroidal binding sites. Precedent exists for this concept. The nonsteroid diethylstilbesterol has been found to manifest all of the properties of steroidal estrogens and has an even higher affinity for the estrogen receptor than estradiol-17 β (33). Additionally, flutamide, another nonsteroid, has been described as a competitive inhibitor of androgen binding to its receptor in ventral prostate (34). The apparent generality of the concept that nonsteroids can bind to steroid receptors as both agonists or antagonists may have wide-spread implications for the future design of pharmacologically active agents.

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REFERENCES

1. Paulus, H. E., and M. W. Whitehouse. 1973. Nonsteroid anti-inflammatory agents. *Annu. Rev. Pharmacol.* **13**: 107-125.
2. Vane, J. R. 1971. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat. New Biol.* **231**: 232-235.
3. Davison, C., and H. G. Mandel. 1971. Nonnarcotic analgesics and antipyretics. I: Salicylates. In *Drill's Pharmacology in Medicine*. J. R. Dipalma, editor. McGraw-Hill Book Co., New York. 4th edition. 379-403.
4. Smith, M. J. H. 1966. Interactions with endocrine systems. In *The Salicylates*. M. J. H. Smith and P. K. Smith, editors. Wiley-Interscience Div., John Wiley & Sons, Inc., New York. Chapter 3. 107-153.
5. Winter, C. A., E. A. Risley, and R. H. Silber. 1968. Antiinflammatory activity of indomethacin and plasma corticosterone in rats. *J. Pharmacol. Exp. Ther.* **162**: 196-201.
6. Domenjoz, R. 1966. Synthetic anti-inflammatory drugs: Concepts of their mode of action. *Adv. Pharmacol.* **4**: 143-217.
7. Brodie, B. B., E. W. Lowman, J. J. Burns, P. R. Lee, T. Chenkin, A. Goldman, M. Weiner, and J. M. Steele. 1954. Observations on the antirheumatic and physiologic effects of phenylbutazone (Butazolidin) and some comparisons with cortisone. *Am. J. Med.* **16**: 181-190.
8. Green, J., and P. O. Williams. 1953. Effect of Butazolidine on the excretion of water and electrolytes. *Lancet*. **I**: 575-577.
9. Hetzel, B. S., J. S. Charnock, and H. Lander. 1959. Metabolic effects of salicylate in man. *Metab. (Clin. Exp.)*. **8**: 205-213.
10. Charnock, J. S., L. J. Opit, and B. S. Hetzel. 1961. Electrolyte distribution in rats following salicylate. *Metab. (Clin. Exp.)*. **10**: 874-882.
11. Elliott, H. C. 1962. Reduced adrenocortical steroid excretion rates in man following aspirin administration. *Metab. (Clin. Exp.)*. **11**: 1015-1018.
12. Ramsay, A. G., and H. C. Elliott. 1967. Effect of acetylsalicylic acid on ionic reabsorption in the renal tubule. *Am. J. Physiol.* **213**: 323-327.
13. Opelz, G., P. I. Terasaki, and A. A. Hirata. 1973. Suppression of lymphocyte transformation by aspirin. *Lancet*. **II**: 478-480.
14. Hofmann, L. M., M. I. Krupnick, and H. A. Garcia. 1972. Interactions of spironolactone and hydrochlorothiazide with aspirin in the rat and dog. *J. Pharmacol. Exp. Ther.* **180**: 1-5.
15. Tweeddale, M. G., and R. I. Ogilvie. 1973. Antagonism of spironolactone-induced natriuresis by aspirin in man. *N. Engl. J. Med.* **289**: 198-200.
16. Gabriel, J. B., H. M. Katz, J. Reiman, and N. M. Luger. 1955. DCA-like effects of Butazolidin in normal subjects and in a patient with Addison's disease. *Metab. (Clin. Exp.)*. **4**: 119-128.
17. Seely, J. R., R. S. Ely, A. K. Done, L. E. Ainger, and V. C. Kelley. 1955. Studies of 17-hydroxycorticosteroids. IX. The influence of therapy on adrenal cortical function in patients with rheumatic fever. *J. Pediatr.* **47**: 434-441.
18. Johnson, H. P., Jr., E. P. Engleman, P. H. Forsham, M. A. Krupp, T. W. Green, and A. Goldfien. 1954. Effects of Phenylbutazone in gout. *N. Engl. J. Med.* **250**: 665-670.
19. Feldman, D., J. W. Funder, and I. S. Edelman. 1972. Subcellular mechanisms in the action of adrenal steroids. *Am. J. Med.* **53**: 545-560.
20. Funder, J. W., D. Feldman, and I. S. Edelman. 1973. The roles of plasma binding and receptor specificity in the mineralocorticoid action of aldosterone. *Endocrinology*. **92**: 994-1004.
21. Funder, J. W., D. Feldman, and I. S. Edelman. 1973. Glucocorticoid receptors in the rat kidney: the binding of tritiated-dexamethasone. *Endocrinology*. **92**: 1005-1013.
22. Warburg, O., and W. Christian. 1942. Isolierung und Kristallisation des Gärungsferments Enolase. *Biochem. Z.* **310**: 384-421.
23. Trinder, P. 1954. Rapid determination of salicylate in biological fluids. *Biochem. J.* **57**: 301-303.
24. Bonsnes, R. W., and H. H. Tausky. 1945. On the colorimetric determination of creatinine by the Jaffe reaction. *J. Biol. Chem.* **158**: 581-591.
25. Funder, J. W., D. Feldman, E. Highland, and I. S. Edelman. 1974. Molecular modifications of anti-aldosterone compounds: Effects on affinity of spiro lactones for renal aldosterone receptors. *Biochem. Pharmacol.* **23**: 1493-1501.
26. Marver, D., J. Stewart, J. W. Funder, D. Feldman, and I. S. Edelman. 1974. Renal aldosterone receptors: Studies with $\{^3\text{H}\}$ aldosterone and the antimineralocorticoid $\{^3\text{H}\}$ spiro lactone (SC-26304). *Proc. Natl. Acad. Sci. U. S. A.* **71**: 1431-1435.
27. Burns, J. J., R. K. Rose, T. Chenkin, A. Goldman, A. Schulert, and B. B. Brodie. 1953. The physiological disposition of phenylbutazone (Butazolidin) in man and a method for its estimation in biological material. *J. Pharmacol. Exp. Ther.* **109**: 346-357.
28. Hucker, H. B., A. G. Zacchei, S. V. Cox, D. A. Brodie, and N. H. R. Cantwell. 1966. Studies on the absorption, distribution and excretion of indomethacin in various species. *J. Pharmacol. Exp. Ther.* **153**: 237-249.
29. Holt, L. P. J., and C. F. Hawkins. 1965. Indomethacin: studies of absorption and of the use of indomethacin suppositories. *Br. Med. J.* **1**: 1354-1356.
30. Quintanilla, A., and R. H. Kessler. 1973. Direct effects of salicylate on renal function in the dog. *J. Clin. Invest.* **52**: 3143-3153.
31. Rousseau, G. G., J. D. Baxter, and G. M. Tomkins. 1972. Glucocorticoid receptors: Relations between steroid binding and biological effects. *J. Mol. Biol.* **67**: 99-115.
32. Reinicke, C. 1975. Influence of non-steroid anti-inflammatory drugs (NSAIDs) on hepatic tyrosine aminotransferase (TA) activity in rats *in vitro* and *in vivo*. *Biochem. Pharmacol.* **24**: 193-198.
33. Korenman, S. G. 1969. Comparative binding affinity of estrogens and its relation to estrogenic potency. *Steroids*. **13**: 173-177.
34. Liao, S., D. K. Howell, and T-M. Chang. 1974. Action of a non-steroidal antiandrogen, flutamide, on receptor binding and nuclear retention of 5 α -dihydrotestosterone in rat ventral prostate. *Endocrinology*. **94**: 1205-1209.