# Effect of Salicylates on Histamine and L-Histidine Metabolism

## INHIBITION OF IMIDAZOLEACETATE PHOSPHORIBOSYL TRANSFERASE

JOEL MOSS, MARIA C. DE MELLO, MARTHA VAUGHAN, and MICHAEL A. BEAVEN

*From the Laboratory of Cellular Metabolism and the Pulmonary Branch, National Heart and Lung Institute, Bethesda, Maryland 20014*

ABSTRACT In man and other animals, urinary excretion of the histidine and histamine metabolite, imidazoleacetate, is increased and that of its conjugated metabolite, ribosylimidazoleacetate, decreased by salicylates. Imidazoleacetate has been reported to produce analgesia and narcosis. Its accumulation as a result of transferase inhibition could play a part in the therapeutic effects of salicylates. To determine the locus of salicylate action, we have investigated the effect of anti-inflammatory drugs on imidazoleacetate phosphoribosyl transferase, the enzyme that catalyzes the ATP-dependent conjugation of imidazoleacetate with phosphoribosylpyrophosphate. As little as 0.2 mM aspirin produced 50% inhibition of the rat liver transferase. In vivo, a 30% decrease in the urinary excretion of ribosylimidazoleacetate has been observed with plasma salicylate concentrations of 0.4 mM. The enzyme was also inhibited by sodium salicylate but not by salicylamide, sodium gentisate, aminopyrine, phenacetin, phenylbutazone, or indomethacin. The last four drugs have been shown previously not to alter the excretion of ribosylimidazoleacetate when administered in vivo. Since both the drug specificity and inhibitory concentrations are similar in vivo and in vitro, it seems probable that the effect of salicylates on imidazoleacetate conjugation results from inhibition of imidazoleacetate phosphoribosyl transferase.

## INTRODUCTION

A principal urinary metabolite of L-histidine (1) and histamine (2–6) in humans and other species is 1-ribosylimidazole-4-acetate, which is formed by conjugation of imidazole-4(5)-acetate with phosphoribosylpyrophosphate in an ATP-dependent reaction (7, 8). In our previous studies (9), administration of therapeutic doses of salicylates in humans resulted in a decrease in the urinary excretion of ribosylimidazoleacetate with a concomitant increase in the excretion of free imidazoleacetate. Because of the reported analgesic and narcotic action of imidazoleacetate (10, 11) and the possibility that salicylates may exert their analgesic effects through alteration in the metabolism of imidazoleacetate, we have investigated further the locus of salicylate action. The work described in this report will show that aspirin, but not the nonsalicylate anti-inflammatory drugs, inhibits the partially purified imidazoleacetate phosphoribosyl transferase, the enzyme that catalyzes the formation of phosphoribosylimidazoleacetate from imidazoleacetate.[1] It appears that salicylates block the metabolism of imidazoleacetate in vivo by inhibiting imidazoleacetate phosphoribosyl transferase.

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[1] The mechanism of conjugation in vivo has not been defined. Crowley (7) has demonstrated that, in the presence of ATP and phosphoribosylpyrophosphate (PRPP), soluble fractions from extracts of rat liver catalyze the formation of phosphoribosylimidazoleacetate (ImARP) from imidazoleacetate (ImA) according to the following reaction.

$$\text{ImA} + \text{ATP} + \text{PRPP} \xrightarrow[\text{phosphoribosyl transferase}]{\text{imidazoleacetate}} \text{ImARP} + \text{ADP} + \text{P}_\text{i} + \text{PP}_\text{i}.$$

The phosphoribosylimidazoleacetate is thought to be converted to the urinary excretion product, ribosylimidazoleacetate, by the action of phosphatase(s). The present finding that salicylates inhibit the conjugation of imidazoleacetate in vivo as well as the above reaction in vitro strongly suggests that the enzyme system described by Crowley is involved in the conjugation in vivo.

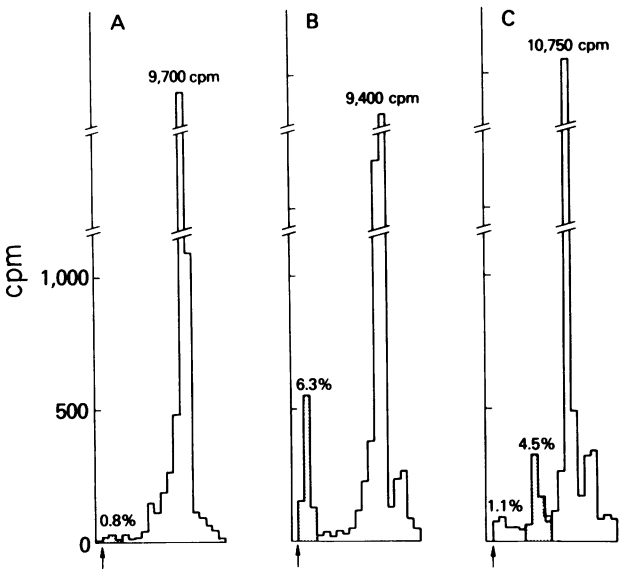


FIGURE 1 Thin layer chromatography of labeled substrate and reaction products in incubations of DEAE-cellulose purified rat liver imidazoleacetate phosphoribosyl transferase. Chromatograms (Solvent A) of: (A) reaction mix without enzyme; (B) reaction mix with enzyme; and (C) reaction mix with enzyme after further incubation with acid phosphatase. Radioactivity was determined in 2.5-mm segments of the chromatogram.

## METHODS

*Materials.* Drugs were obtained from the following suppliers: Acetylsalicylic acid powder USP, Mallinckrodt Inc., St. Louis, Mo.; sodium salicylate, USP, Aldrich Chemical Co., Inc., Milwaukee, Wisc.; phenylbutazone, Geigy Pharmaceuticals, Ardsley, N. Y.; phenacetin, Eastman Kodak Co., Rochester, N. Y.; indomethacin, Merck Sharp & Dohme, Rahway, N. J.; salicylamide and aminopyrine, Gilman Inc., Washington, D. C.; and sodium gentisate (2,5-dihydroxybenzoic acid, sodium salt), Nutritional Biochemicals Corp., Cleveland, Ohio. Solutions of the drugs were adjusted to pH 7.4 before use. Reagents were obtained from the following sources: 5-phosphorylribose-1-pyrophosphate dimagnesium dihydrate, Boehringer Mannheim Corp., Biochemical Div., New York; ATP, phosphoenol pyruvate, Trizma base, and ribose-5-phosphate, Sigma Chemical Co., St. Louis, Mo.; EDTA and potassium phosphate from Fisher Scientific Co., Pittsburgh, Pa.; sodium dodecyl sulfate from Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.; and DE 23 (DEAE-cellulose from Whatman Chemicals, Div. W & R Balston, Maidstone, Kent, England). The DEAE was equilibrated by washing 250 g with 0.5 M NaOH, water (until neutral); 0.5 M HCl, water (until neutral); 0.5 M potassium phosphate, pH 7.0; and 5 mM potassium phosphate, 1 mM EDTA, pH 7.0 (final volume 2 liters). Polygram Cel, thin layer cellulose-coated (0.1-mm) plastic sheets (5 × 20 cm) were purchased from Macherey-Nagel and Co., Düren, W. Germany.

[$^3$H]Imidazoleacetic acid, 1.3 Ci/mmol, prepared from unlabeled imidazoleacetic acid by catalytic exchange with tritium gas, was purified by thin layer chromatography (9) and stored in solution, 1 mCi per ml of 0.01 N HCl, at −20°C. 2(Ring carbon)-[$^{14}$C]histamine (52 mCi/mmol), was purchased from Amersham/Searle Corp., Arlington Heights, Ill. Unlabeled histamine metabolites were obtained from sources described earlier (9). Acid phosphatase (potato, grade B) was obtained from Calbiochem, San Diego, Calif. and pyruvate kinase from Sigma Corporation, San Diego, Calif.

*Purification of imidazoleacetate phosphoribosyl transferase.* Male Osborne Mendel rats were anesthetized with ether. A midline abdominal incision was made, the portal vein catheterized, and the inferior vena cava severed. The portal vein was flushed with 0.25 M sucrose containing 50 mM Tris ($Cl^-$), pH 7.5. After decapitation of the rat, the liver was excised, minced finely with a razor blade, and homogenized (10 strokes) with a tight-fitting ground-glass homogenizer in 5 volumes of 20 mM potassium phosphate, 5 mM $MgCl_2$, 1 mM EDTA, pH 7.5, at 0–4°C. The homogenate was filtered through two layers of cheesecloth and centrifuged at 100,000 *g* for 40 min. The supernatant fraction was stirred with an equal volume of DEAE-cellulose which had been previously equilibrated with 5 mM potassium phosphate, 1 mM EDTA, pH 7.0. After centrifugation at 5,000 *g* for 30 min, the supernatant fraction was discarded. The DEAE-cellulose was then washed three times by stirring with 5 volumes of the same buffer for 30 min. The washed DEAE-cellulose was extracted with 5 volumes of 1 mM EDTA in 0.5 mM potassium phosphate buffer, pH 7.0, for 30 min and the extract centrifuged at 5,000 rpm for 10 min to remove cellulose fibers. The enzyme was prepared on the day of the experiment.

*Assay of imidazoleacetate phosphoribosyl transferase.* The standard assay mixture contained 0.4 mM imidazoleace-

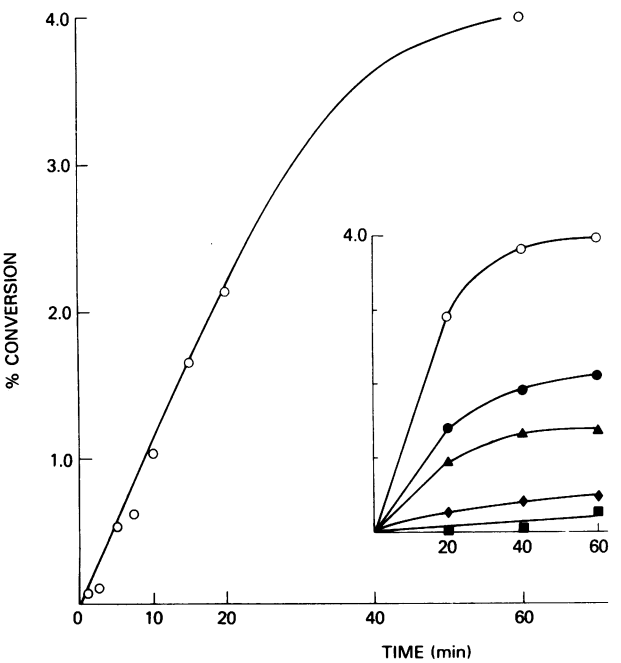


FIGURE 2 Time course of conversion of [$^3$H]imidazoleacetate to [$^3$H]phosphoribosylimidazoleacetate by the rat liver imidazoleacetate phosphoribosyl transferase and the effect of aspirin. Each assay contained 0.5 mg of DEAE-cellulose purified enzyme and, as indicated in the inset, (○) no aspirin, (●) 0.12 mM, (▲) 0.25 mM, (◆) 1.25 mM, and (■) 2.5 mM aspirin.

tate, [$^3$H]imidazoleacetate (1 $\mu$Ci), 0.8 mM phosphoribosylpyrophosphate, 0.3 mM ATP, 10 mM $MgCl_2$, and 120 mM Tris ($Cl^-$), pH 8.15, in a final volume of 0.2 ml. All assay components except enzyme were incubated at 37°C in 0.1 ml for 3 min. Assays were then started by the addition of 0.1 ml of enzyme in 0.5 M potassium phosphate, 1 mM EDTA, pH 7. Unless otherwise stated, after incubation for 20 min at 37°C, assays were terminated by the addition of 0.3 ml of a solution of 1% sodium dodecyl sulfate. The reaction product [$^3$H]phosphoribosylimidazoleacetate was separated from [$^3$H]imidazoleacetate by thin layer chromatography. Duplicate samples (10 $\mu$l) of the incubation mixture were applied as 2 × 40-mm bands on thin layer cellulose sheets, which were developed for 120 min in butanol : ethanol : concentrated ammonia, 8 : 1 : 3 parts by volume (solvent A). The plates were dried in air and 2.5-mm wide sections of the chromatogram scraped into glass vials for radioassay by liquid scintillation spectrometry as described earlier (9). The radioactivity in the area corresponding to phosphoribosylimidazoleacetate (fractions 1–4) was determined. Transferase activity is reported as "percent conversion" (dpm [$^3$H]phosphoribosylimidazoleacetate ÷ dpm [$^3$H]imidazoleacetate × 100) and is the average of duplicate assays.

*Further identification of reaction product(s).* After incubation of the standard assay mixture without enzyme for 20 min, essentially all (> 97%) of the radioactivity migrated as imidazoleacetate and < 1% was recovered in fractions 1–4 (Fig. 1A). After incubation with enzyme, a new radioactivity peak was observed in fractions 1–4 and corresponded to 6.3% of the tritium (Fig. 1B). As no standard phosphoribosylimidazoleacetate was available, indirect evidence for the identity of the labeled material in fractions 1–4 was obtained by demonstrating that on treatment with acid phosphatase most of the labeled material was converted to [$^3$H]-ribosylimidazoleacetate (Fig. 1C). The latter product had an $R_F$ identical to that of authentic [$^3$H]ribosylimidazoleacetate (generally labeled) and 1-ribosylimidazole[2-$^{14}$C]4-acetate isolated by chromatography from urine of rats that had received [$^3$H]imidazoleacetate or [2-(ring carbon)-$^{14}$C]-histamine intravenously (9). The labeled product obtained after phosphatase treatment co-chromatographed with [$^{14}$C]-ribosylimidazoleacetate in solvent A, in *n*-butanol : acetic acid : water (12 : 3 : 5) and in *n*-butanol : pyridine : water (1 : 1 : 1).

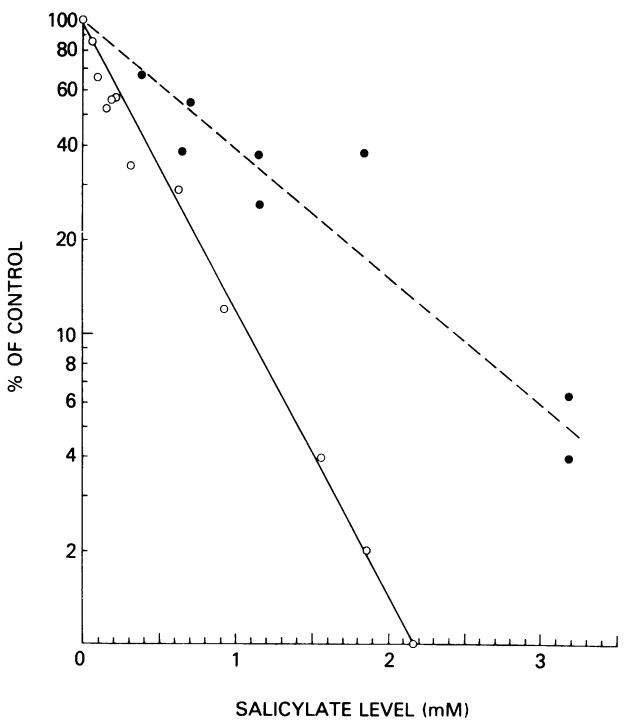


FIGURE 3 Comparison of the effects of aspirin on ribosyl conjugation in vivo and in vitro. (●), inhibition of excretion of [$^3$H]ribosylimidazoleacetate with the indicated plasma concentrations of aspirin, calculated from data in Ref. 9; (○), inhibition of activity of the DEAE-cellulose-purified imidazoleacetate phosphoribosyl transferase with the indicated concentrations of aspirin. The concentration causing 50% inhibition in vivo was 0.7 mM and in vitro ~ 0.2 mM.

TABLE I

*Effect of Aspirin on the Formation of [$^3$H]Phosphoribosylimidazoleacetate by the Crude Rat Liver Supernatant Enzyme Preparation with and without PRPP and ATP Regeneratjng Systems*

| Additions | Transferase activity: No aspirin | Transferase activity: 1 mM aspirin |
|---|---|---|
| | *% conversion* | |
| None | 0.3 | 0.3 |
| Ribose-5-phosphate (R-5-P) | 1.5 | 0.5 |
| Pyruvate kinase (PK) | 0.3 | <0.1 |
| Phosphenol pyruvate (PEP) | 1.6 | 0.6 |
| PK + PEP | 1.7 | 0.6 |
| R-5-P + PK + PEP | 5.3 | 1.3 |

In addition to the standard assay components, incubations contained rat liver supernatant fraction (940 $\mu$g of protein). Where indicated, components of an ATP-regenerating system (16.7 mM phosphenol pyruvate and 16 $\mu$g of pyruvate kinase) and 5 mM ribose-5-phosphate (which served as substrate for the phosphoribosylpyrophosphate synthetase present in the supernatant fraction) were also present.

## RESULTS

*Inhibition by aspirin of the conversion of [$^3$H]imidazoleacetate to [$^3$H]phosphoribosylimidazoleacetate by imidazoleacetate phosphoribosyl transferase.* The activity of the DEAE-cellulose-purified enzyme was constant under standard assay conditions for 20 min and was inhibited significantly by therapeutic concentrations of aspirin (Fig. 2). The inhibition was proportional to the concentration of aspirin: ~ 0.2 mM aspirin caused 50% inhibition of the transferase. Beaven et al. (9) observed 30% inhibition of ribosylimidazoleacetate excretion in vivo with plasma concentrations of 0.38 mM and 50% inhibition with 0.7 mM aspirin (Fig. 3). Purification of the transferase beyond the initial centrifugation step was not required to demonstrate the effect of aspirin on enzymatic activity. With crude transferase preparations, however, addition of ribose-5-phosphate or an ATP

TABLE II

*Effect of Salicylates and Other Drugs on the Conversion of [$^3H$]Imidazoleacetate to [$^3H$]Phosphoribosylimidazoleacetate by DEAE-Cellulose-Purified Imidazoleacetate Phosphoribosyl Transferase*

| Exp. no. | Additions | Concentration | Transferase activity: Conversion | Transferase activity: Control |
|---|---|---|---|---|
| | | *mM* | % | |
| 1 | Vehicle* | — | 3.7 | 100 |
| | Aspirin | *0.1* | 2.4 | 65 |
| | | *0.5* | 1.4 | 38 |
| | Sodium salicylate | *0.1* | 2.2 | 59 |
| | | *0.5* | 1.5 | 49 |
| | Sodium gentisate | *0.1* | 3.7 | 100 |
| | | *0.5* | 3.5 | 95 |
| | Salicylamide | *0.1* | 3.6 | 97 |
| | | *0.5* | 3.1 | 84 |
| | Phenacetin | *0.1* | 3.6 | 97 |
| | | *0.5* | 3.8 | 100 |
| | Aminopyrine | *0.1* | 3.8 | 100 |
| | | *0.5* | 3.7 | 100 |
| 2 | Vehicle‡ | — | 2.0 | 100 |
| | Phenylbutazone | *0.1* | 2.5 | 125 |
| | | *0.5* | 2.9 | 145 |
| | Indomethacin | *0.1* | 2.5 | 125 |
| | | *0.5* | 2.9 | 145 |

Assays containing the standard reaction mixture, partially purified imidazoleacetate phosphoribosyl transferase, 0.5 mg of protein, and drugs as indicated were incubated for 20 min.

* Water, 10 μl. In experiment 1, drugs were added in 10 μl of water (pH adjusted to 7.4 with 0.1 N NaOH).

‡ Ethanol, 10 μl. In experiment 2, the drugs were added in 10 μl ethanol. Ethanol alone inhibited the formation of [$^3$H]phosphoribosylimidazoleacetate.

regenerating system was necessary to observe formation of the ribosylated product. When both were present the yield of phosphoribosylimidazoleacetate was increased. The inhibition of transferase activity by aspirin was apparent, whether the various components of the regenerating systems were added singly or in combination (Table I).

*Effect of salicylates and other nonsalicylate anti-inflammatory drugs.* Significant inhibition of the transferase was noted with aspirin and sodium salicylate, both of which were found to be effective inhibitors of ribosylimidazoleacetate excretion in vivo (9). The salicylate derivative, salicylamide, which did not decrease ribosylimidazoleacetate excretion in vivo, did not inhibit the transferase reaction in vitro (Table II). The salicylate metabolite, sodium gentisate, was also without effect on the transferase. Nonsalicylate anti-inflammatory drugs, such as phenacetin, aminopyrine, phenylbutazone, and indomethacin, which were inactive in vivo, did not inhibit the reaction in vitro (Table II).

## DISCUSSION

The question arises whether inhibition of imidazoleacetate phosphoribosyl transferase is metabolically significant in humans after therapeutic doses of salicylates. Our original studies (9) showed that 80–85% inhibition of the urinary excretion of ribosylimidazoleacetate occurred in patients receiving three 300-mg aspirin tablets four times a day. In additional studies, substantial inhibition (~80%) of ribosylimidazoleacetate excretion was noted in subjects receiving 600 mg of aspirin four times a day.[2] Although studies have not been conducted after single doses of salicylates, it is known that plasma salicylate levels of about 0.5 mM are achieved after two 600-mg aspirin tablets (see, for example, 12). Since it has been shown that in several species salicylate levels in liver and kidney, two major sources of imidazoleacetate phosphoribosyl transferase (7), are approximately the same as those in plasma (13–15) and since 50% inhibition of the transferase is observed with 0.25 mM (~5 mg/100 ml) salicylate (Fig. 2), it seems likely that ribose conjugation would be impaired by therapeutic doses of salicylates.

TABLE III

*Comparison of the Effect of Salicylates on Biological Systems*

| Inhibition of | $ID_{50}$* | Reference no. |
|---|---|---|
| | *mM* | |
| Prostaglandin synthetase, dog spleen | 0.04 | 16, 18 |
| rabbit brain | 0.06 | 17, 18 |
| seminal vesicle | 5.5 | 19 |
| Phosphoribosylimidazoleacetate transferase | 0.2–0.3 | This study |
| Ribosylimidazoleacetate formation in vivo | 0.7 | 9 |
| [$^3$H]Leucine incorporation into rat liver mitochondrial-microsomal protein, in vitro | 0.6‡ | 20 |
| [$^3$H]Thymidine incorporation into DNA-human leukocytes | 0.4–0.5§ | 21 |
| Bacterial growth | 0.25–0.5§ | 22 |

* Concentration required for 50% inhibition unless otherwise stated.

‡ Caused 66% inhibition.

§ Values estimated from published data.

Aspirin also inhibits prostaglandin synthetases from many species and tissues (Table III). The $ID_{50}$ of aspirin found with the prostaglandin synthetase from various preparations varied from one-fifth to more than 20 times that observed for the liver transferase in the present study. As noted above, nonsalicylate agents such as indomethacin and phenylbutazone, which are very effective inhibitors of prostaglandin synthetase (16, 18), had no effect on imidazoleacetate phosphoribosyl transferase (Table II). Aspirin is a potent inhibitor of plate-

[2] Beaven, M. A., Z. Horakova, and H. Keiser. 1976. *Experientia (Basel)*. In press.

let aggregation. This aspirin effect apparently results from the irreversible acetylation of a specific platelet protein (23). Sodium salicylate inhibits platelet aggregation only at concentrations several thousand times those at which aspirin is effective (24). Since aspirin and sodium salicylate were equipotent in inhibiting the transferase, acetylation is presumably not involved in the inhibition. Aside from its effects on platelet aggregation and the transferase, aspirin has several other inhibitory actions (Table III). Among these are inhibition of (*a*) protein synthesis by rat liver preparations (20), (*b*) protein and DNA synthesis during blast transformation of human lymphocytes (21, 25, 26), and (*c*) bacterial growth (22). The $ID_{50}$ for aspirin in the transferase reaction was of the same magnitude as those noted for (*a*), (*b*), and (*c*), (Table III).

The effect of aspirin on L-histidine metabolism may be of clinical importance, since this amino acid is the principal source of imidazoleacetate (1). Although it has often been assumed that imidazoleacetate is pharmacologically inactive (see, for example, 27), it does have pronounced central effects. In the mouse, which conjugates imidazoleacetate poorly,[2] imidazoleacetic acid, in doses of 200 μg/g intraperitoneally, produces prolonged narcosis and analgesia (10, 11; and confirmed in this laboratory). The analgesic effects of salicylates may in part be due to an accumulation of imidazoleacetate, although this has not been determined as yet.

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