Comparison of Antipyretic Activity of Salicylic and Aurintricarboxylic Acids.* (28106)

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(Introduced by Robert L. Straube)
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Salicylates and related compounds, especially the dye aurintricarboxylic acid (ATA), are effective antidotes in experimental beryllium poisoning(1,2) primarily because of their chelating properties(3). ATA is more effective than the salicylates with respect both to dosage and time relationships(4). It also shares with salicylic acid the ability to control accelerated blood sedimentation rate(5). The present paper compares sodium salicylate and ATA with respect to another therapeutic property characteristic of the salicylates, antipyresis.

Materials and methods. Mature female Sprague-Dawley rats, maintained in an air-conditioned room at 22°, were used. Fevers were produced by subcutaneous injection of 3.0 ml of a 15% yeast suspension which was prepared immediately before use by mixing baker's active dry yeast in 0.85% NaCl solution. Sodium salicylate and the ammonium salt of ATA were injected as aqueous solutions at about pH 7.2. They were injected intravenously about 18 hours after yeast administration when the induced fever was still at a maximum level. Freshly boiled, double-distilled water was used in preparation of all solutions.

Rectal temperatures were measured with a rapid-recording Schultheis mercury thermometer. In each experiment 25 rats were sorted into 5 groups of 5 rats each. Each group contained rats with a representative range of control temperatures; mean temperatures of all groups agreed within 0.1°. The rats of 4 groups were then injected with yeast and those of the fifth with saline. Before treatment temperatures of the rats were again measured and, if necessary to equate the group mean temperatures, 1 or 2 rats were exchanged between "fevered" groups. Three of the groups with fevers were injected with antipyretic compounds, the fourth with saline. The fifth, the saline control group, again received saline. In experiments designed to evaluate the effects of antipyretics on normal temperature, the rats were sorted into groups of 5 on the basis of their normal temperatures as in the initial sorting described above and then were given the test substance or saline intravenously.

Results and discussion. Body temperatures in a population of 651 uninjected stock rats ranged from 36.6 to 39.8° with a normal frequency distribution and a mean of 38.05° ± 0.02. The temperatures rose within a few hours after injection of yeast and, between 5 and over 29 hours, remained at a plateau about 1° above those of the saline-injected controls. Sixteen to 18 hours after yeast administration, just before treatment was given, the mean temperature was 38.98° ± 0.01.

Both sodium salicylate and ATA reduced this fever. The doses required were similar, although on a molar basis smaller amounts of ATA were effective (Fig. 1). The fever was reduced about 1.0-1.1° by 125 mg/kg of either substance. Lowering the dose below 100 mg/kg gave a proportionally decreasing effect.

Differences were observed, however, in the action of the 2 compounds with regard to duration of effect, ability to reduce temperature significantly below the mean of the controls, and degree of antipyresis as related to the degree of fever. ATA produced its maximal effect by 1 hour after injection but its effect decreased rapidly thereafter and had disappeared by 5 hours. The salicylate effect was maintained essentially unchanged from 1 through 3½ hours, and was still apparent at 5 hours (Fig. 2). When fully effective doses of the 2 compounds were injected into the

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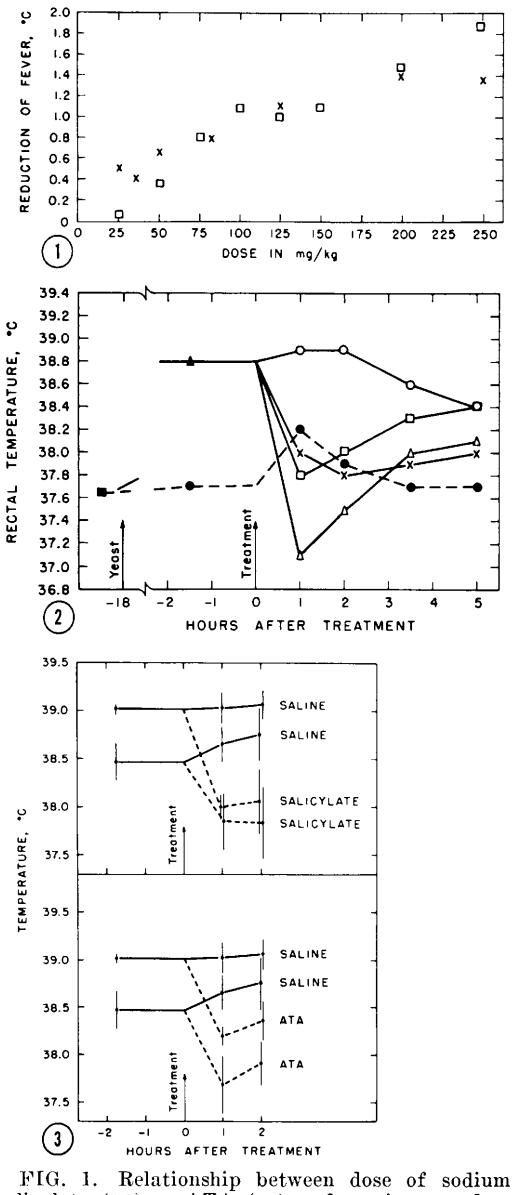

FIG. 1. Relationship between dose of sodium salicylate (×) or ATA (□) and maximum reduction of fever. The points in the dose range of 50 to 125 mg/kg represent mean values from 10 to 30 rats per point as compared with an equal number of saline controls; all others, 5 to 10 rats.

FIG. 2. Typical experiment demonstrating effect of ATA and sodium salicylate on temperatures of rats with yeast-induced fevers. Symbols: □, ATA, 125 mg/kg; ×, sodium salicylate, 125 mg/kg; △, ATA plus salicylate, 125 mg/kg of each, the salicylate being given immediately after the ATA; ●, unfevered controls; ○, fevered controls; ■, all animals; ▲, all animals with fevers. Treatment was given 18½ hr after the yeast injection. Controls received equivalent volumes of saline. Each group was composed of 5 rats.

FIG. 3. Comparison of antipyretic effects of 125 mg/kg of sodium salicylate (top) or of ATA (bottom) on "high" and "low" fevers. Treatment was administered 20 hr after yeast injection. Each point represents the mean temperature of 10 rats, or of 7 and 8 in the salicylate-treated groups. The deviation from the mean shown by the vertical lines is 2 times standard error of mean.

same rat their effects on fever at 1 hour were essentially additive, while each drug appeared to exert its typical action on the temperature curve (initial steep drop with ATA, continued maintenance of effect with salicylate) (Fig. 2). Additive effects were also observed in experiments in which partially effective doses of each drug were used.

As expected, sodium salicylate, given in single doses up to 250 mg/kg, did not reduce the body temperature of either normal or yeast-injected rats significantly below the level of saline-injected controls. ATA, on the other hand, did reduce the mean temperature of normal rats for about 1 hour. Doses of 125 to 175 mg/kg lowered body temperature 0.7-1.4° below that of saline controls (50 rats; $P<0.01>0.001$) and 250 mg/kg reduced it by 1.9° (20 rats; $P<<0.001$). In rats with fevers, however, a dose of 250 mg/kg of ATA was required to lower the temperature below that of saline controls. The significance of this is questionable, since the presence of diarrhea in this group indicated that the combined effect of this dose of ATA and the yeast may have been slightly debilitating. These reductions never resulted in individual temperatures below the lowest normal recording of 36.6°.

Since the amount of fever developed after yeast administration was proportionally greater in rats with lower initial temperatures, the relationship between degree of antipyresis and magnitude of the fever was investigated. The yeast-injected rats were in this case divided into 2 groups, those with high and low fevers (mean temperatures 39.0 and 38.5°, respectively). Sodium salicylate reduced the fever, whether it was initially high or low, to the same temperature whereas ATA reduced it by a specific decrement (Fig. 3). Thus for 2 hours after ATA treatment rats with high and low initial fevers remained statistically distinct groups ($P<0.01>0.001$). The temperatures of control rats with high

and low fevers remained different for 1 hour after saline injection ($P<0.01>0.001$), while those given salicylate were the same ($P>>0.05$).

Thus, although both ATA and sodium salicylate reduced the experimental fever, their actions were somewhat different. ATA had a less lasting effect, reduced the temperature of rats without fevers below the mean for normal rats, and reduced fever by a specific amount whereas salicylate reduced it to a specific temperature. A tentative explanation which reconciles these differences is that the principal action of ATA is peripheral and direct in contrast to the effect of salicylate which is probably mediated through the central nervous system(6). Salicylate has been shown to cross the blood-brain barrier(7), but ATA, because of the size of the particles it forms in the body and because of its attachment to tissue proteins, probably cannot do so. Both the ability of the dye to form lakes *in vivo*(1,8) and its high protein affinity(9,10)‡ lead to the formation of large aggregates in the blood, while its combination with tissue protein results in lasting retention at the sites of deposition(8). Reduction of temperature by direct action on peripheral systems, as suggested for ATA, would presumably be less regulated than action mediated through the central nervous system. As a result body temperature would be lowered below normal, as it was to a limited extent by ATA, and would be lowered by a specific amount, independent of the degree of fever. The peripheral systems that may be acted upon by ATA to effect heat loss are not obvious, although the piloerection generally noted in yeast-injected rats disappeared after ATA (and also salicylate) administration. No increase in salivation or in peripheral vasodilation was evident following either compound in these experiments.

‡ For example, the binding constant of ATA with albumin is 48,000, compared to 1,920 for salicylate (10).

Summary. The antipyretic effects of salicylic acid and of a structurally related compound, the dye aurintricarboxylic acid (ATA), were compared in rats with fevers induced by subcutaneous injection of yeast. The two compounds had comparable effects on fever and, when injected together, their effects were additive. ATA had a more transient effect, reduced the temperature of rats without fevers, and reduced fevers by a specific amount rather than to a specific temperature as did salicylate. These differences led to the postulation of a direct peripheral action of ATA.

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