Competitive Antagonism of Leukocytic Pyrogen by Sodium Salicylate and Acetaminophen[1] (36849)

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The antipyretic action of non-narcotic analgesics is commonly attributed to their ability to "reset the thermostat" back toward normal after the setting has been elevated by a pyrogen (1). This concept implies that the antipyretics act on the thermostat *per se* independently of the pyrogen. Such an action is perhaps most likely with agents such as pyrazolones and para-aminophenol derivatives when they are given in doses large enough to cause hypothermia in afebrile animals (2). However, in the case of salicylates, which lower body temperature only in febrile states (3–5), an action to antagonize the pyrogen at a receptor site seems more likely, with the set point lowering being secondary to the diminished effectiveness of the pyrogen. A number of authors (6–8) have suggested a competitive antagonism between the antipyretic and pyrogen, but there have been no reports of experiments specifically designed to examine the type of inhibition. Salicylates and acetaminophen given peripherally or centrally inhibit leukocytic pyrogen (LP)-induced fever (7–13). Since too frequent administration of salicylate peripherally can lead to cumulation in the cat (14), we have examined the effect of centrally administered sodium salicylate and acetaminophen on the log dose–response curves to LP. The results are compatible with competitive antagonism of this pyrogen by both antipyretics in the doses used.

Methods. Adult mongrel cats were used. Procedures for care and feeding of the animals, for recording body temperature chronically from the retroperitoneal space, for making iv or lateral cerebral ventricular injections, for collection of LP from blood leukocytes, for sterilization of glassware, and for otherwise avoiding contamination have been described previously (12, 15). Ambient temperature was maintained at 22 ± 1.5°. Injections of the antipyretics in saline solution or of saline solution alone were given intraventricularly at 9:30 AM ± 5 min in a volume of 0.10 ml. LP was injected iv 30 min later and was flushed in with 1.0 ml of saline solution. The average of temperature readings at 9:00, 9:15, and 9:30 AM was used as the base line from which changes were measured. Instead of plotting responses on graph paper and determining the area between the curve and the base line with a planimeter as was done previously (13) to obtain a "thermal response index" (TRI), individual responses at approximately 3-min intervals were tabulated, and the comparable area was determined with a calculator such that 1 unit of TRI is equivalent to a 1° change lasting for 1 hr. TRI's were determined for the time period between LP injection and recovery of body temperature to base line. Maximum elevation in temperature above the base line was also determined for each response.

Preliminary injections of LP were given to each animal to determine a volume which produced fevers lying at approximately the midpoint of the linear portion of the log dose–response curve as determined from a previous study (12). This optimal volume of extract was then arbitrarily considered to equal 2 units of LP for that cat. Five cats were used for each study. In a randomized, cross-over order at daily intervals, each cat was given doses of 1, 2, and 4 units of LP iv preceded by saline solution intraventricularly and 2, 4, and 8 units of LP preceded by a

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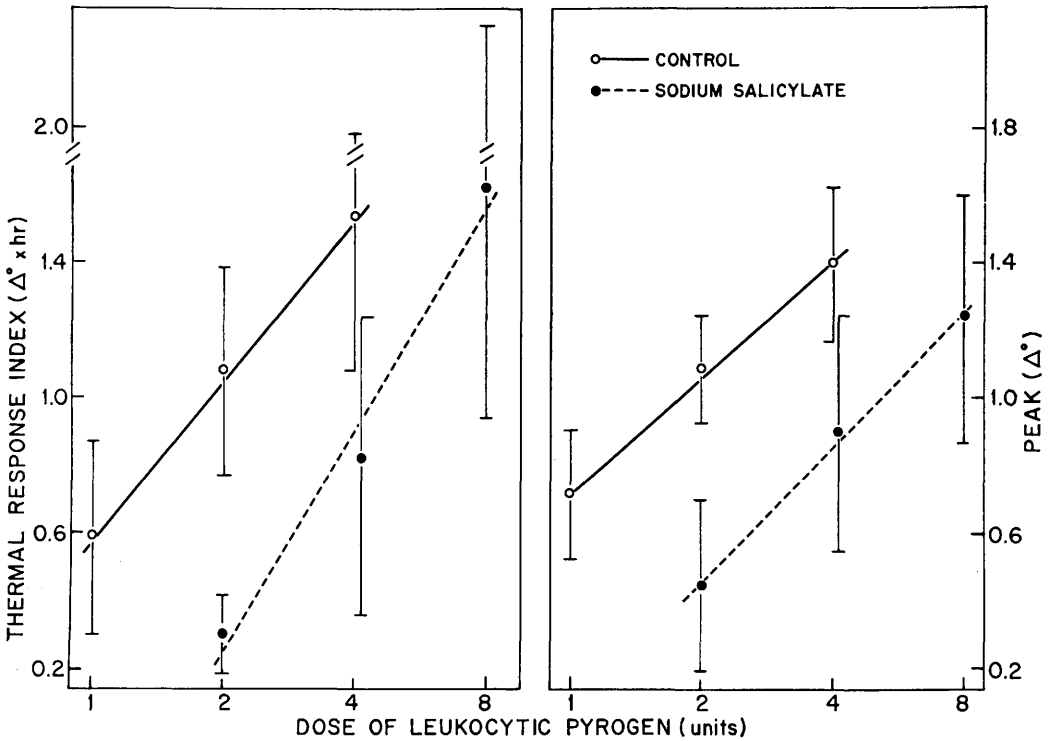

FIG. 1. Antipyretic effect of 1.00 mg of sodium salicylate determined in a cross-over study using 5 cats. The left and right panels indicate thermal response indexes and peak responses, respectively. The positions of the lines were determined by the method of least squares. Mean values and 95% confidence limits are also shown.

dose of antipyretic estimated to reduce the potency of LP approximately 50% (13). Antipyretic was flushed from the ventricular cannula in the afternoon. The data was evaluated by analysis of variance, and potency ratios were determined according to Finney (16).

Results. Two assays were done with sodium salicylate and one with acetaminophen. In the initial assay, which is not illustrated, 0.50 mg of sodium salicylate caused a parallel shift to the right of the log dose–response curve to LP and reduced the potency of LP approximately one-third (Table I). Results of assays using 1.00 mg of sodium salicylate and 0.50 mg of acetaminophen are shown in Figs. 1 and 2, respectively, and in Table I. The corresponding analyses of variance are given in Table II. As shown, similar results were obtained with either TRI or maximum temperature increase as the dependent variable. In each of the three assays highly significant regressions were obtained, but there were no significant deviations from linearity or parallelism.

Discussion. A number of recent reports (4, 8–11, 13) have documented inhibition by centrally administered salicylates and acetaminophen of fever induction by LP, an endogenous product thought to be the ultimate cause of a wide variety of fevers through a central action. The parallel shift to the right of the log dose–response curve to LP pro-

TABLE I. Potency Ratios.

Antipyretic	Dose (mg)	TRI	Peak
Sodium salicylate	0.50	0.67[a] (0.46–0.96)	0.63 (0.47–0.84)
	1.00	0.41 (0.30–0.57)	0.33 (0.25–0.46)
Aceta-minophen	0.50	0.46 (0.31–0.66)	0.41 (0.34–0.51)

[a] Ratio (95% confidence limits).

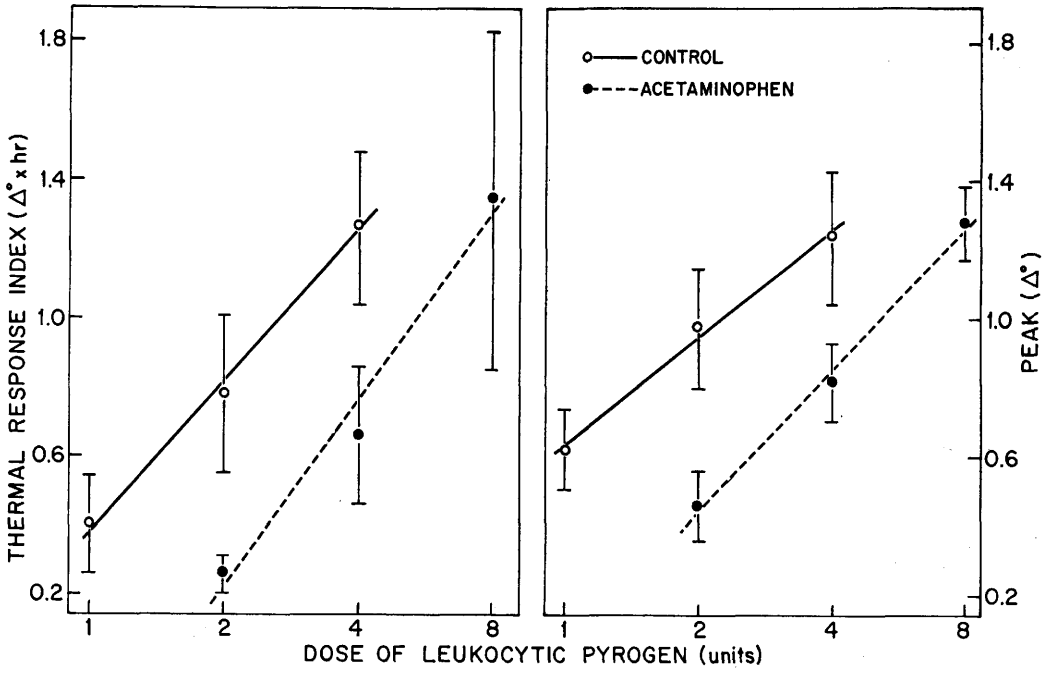

FIG. 2. Antipyretic effect of 0.50 mg of acetaminophen. The data are expressed as in Fig. 1.

duced by pretreatment with sodium salicylate and acetaminophen in the present experiments may indicate that their antipyretic effects were due to competitive antagonism of LP at receptor sites within the central nervous system, most likely in the preoptic/anterior hypothalamus and midbrain where local administration of salicylates has been reported to antagonize LP (8, 11). Since the structures of these antipyretics are so dissimilar from that of LP, which is a small protein (17), it is more likely that the antagonism was a case of allosteric inhibition giving the appearance kinetically of competitive antago-

TABLE II. Analyses of Variance of Assays of Antipyretic Activity of Sodium Salicylate and Acetaminophen.

Source of variation	*df*	Sodium salicylate, 1.00 mg				Acetaminophen, 0.50 mg			
		TRI		Peak		TRI		Peak	
		MS	F	MS	F	MS	F	MS	F
Error	20	0.1178		0.0395		0.1118		0.0190	
Between cats	4	0.2858	2.42	0.1597	4.04[b]	0.0170	<1	0.0627	3.30[d]
Between doses	5	1.3672	11.60[a]	0.6171	15.61[a]	0.9888	8.84[a]	0.5473	28.77[a]
Regression	1	6.4158	54.45[a]	2.7390	69.29[a]	4.7890	42.82[a]	2.5985	136.58[a]
Parallelism	1	0.1767	1.50	0.0176	<1	0.0633	<1	0.0506	2.66
Preparations	1	0.1763	1.50	0.3245	8.21[c]	0.0295	<1	0.0730	3.84
Linearity	2	0.0336	<1	0.0021	<1	0.0311	<1	0.0069	<1

[a] $p < .001$.
[b] $p < .025$.
[c] $p < .01$.
[d] $p < .05$.

nism (18) than that nearly identical attachments to the same receptors were involved. Although 0.50 mg doses of acetaminophen produced small but statistically significant reductions in the body temperatures of afebrile animals (13), there was no indication from the shift of the dose–response curve in this study of an action other than antagonism of the pyrogen. Acetaminophen appeared to be somewhat more potent than sodium salicylate. Previously, however, a 0.25 mg dose of sodium salicylate produced statistically significant antipyresis against LP while the same dose of acetaminophen did not (13). Thus when given intraventricularly these two antipyretics must be very nearly equal in potency in the cat.

Summary. Bioassays of the pyrogenic activity of LP in the presence and absence of antipyretics were performed in cats. Sodium salicylate (0.50 and 1.00 mg) and acetaminophen (0.50 mg) given intraventricularly 30 min before iv injection of LP produced parallel shifts to the right of the log dose–response curve to LP. Such shifts are compatible with the hypothesis that these agents compete with LP for receptor sites within the central nervous system.

1. Woodbury, D. M., *in* "The Pharmacological Basis of Therapeutics" (L. S. Goodman and A. Gilman, eds.), 4th ed., p. 314. Macmillan, New York (1970).

2. Borison, H. L., and Clark, W. G., *in* "Advances in Pharmacology" (S. Garattini and P. A. Shore, eds.), Vol. 5, p. 129. Academic Press, New York (1967).

3. Rosendorff, C., and Cranston, W. I., Clin. Sci. **35**, 81 (1968).

4. Cranston, W. I., Luff, R. H., Rawlins, M. D., and Rosendorff, C., J. Physiol. (London) **208**, 251 (1970).

5. Clark, W. G., J. Pharmacol. Exp. Ther. **175**, 469 (1970).

6. Wit, A., and Wang, S. C., Amer. J. Physiol. **215**, 1160 (1968).

7. Rawlins, M. D., Rosendorff, C., and Cranston, W. I., *in* "Pyrogens and Fever" (G. E. W. Wolstenholme and J. Birch, eds.), p. 175. Churchill Livingstone, Edinburgh (1971).

8. Lin, M. T., and Chai, C. Y., J. Pharmacol. Exp. Ther. **180**, 603 (1972).

9. Chai, C. Y., Lin, M. T., Chen, H. I, and Wang, S. C., Neuropharmacology **10**, 715 (1971).

10. Cranston, W. I., Luff, R. H., Rawlins, M. D., and Wright, V. A., Brit. J. Pharmacol. **41**, 344 (1971).

11. Cranston, W. I., and Rawlins, M. D., J. Physiol (London) **222**, 257 (1972).

12. Clark, W. G., and Moyer, S. G., J. Pharmacol. Exp. Ther. **181**, 183 (1972).

13. Clark, W. G., and Alderdice, M. T., Proc. Soc. Exp. Biol. Med. **140**, 399 (1972).

14. Davis, L. E., and Donnelly, E. J., J. Amer. Vet. Med. Ass. **153**, 1161 (1968).

15. McCarthy, L. E., and Borison, H. L., Anat. Rec. **155**, 305 (1966).

16. Finney, D. J., "Statistical Method in Biological Assay," 2nd ed., 668 pp. Hafner, New York (1964).

17. Snell, E. S., and Atkins, E., *in* "The Biological Basis of Medicine" (E. E. Bittar and N. Bittar, eds.), Vol. 2, p. 397. Academic Press, New York (1968).

18. Goldstein, A., Aronow, L., and Kalman, S. M., "Principles of Drug Action: The Basis of Pharmacology," 884 pp. Harper & Row (Hoeber), New York (1968).

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