Effects of Sodium Acetylsalicylate on the Release of Pyrogen from Leukocytes[1] (36319)

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Acetylsalicylic acid (aspirin) has been the most commonly used antipyretic in clinical medicine. Its mechanisms of antipyresis remain to be elucidated.

It has been reported that sodium acetylsalicylate released neurons in the anterior hypothalamus from inhibition after administration of bacterial endotoxin (1). In animals made pyrexic by leukocytic pyrogen, a much smaller dose of sodium acetylsalicylate was required to reduce the febrile reaction when the drug was administered intracerebroventricularly compared with other routes of administration (2). These observations provide evidence that supports a central locus of action of acetylsalicylate for antipyresis.

Other investigators have presented experimental findings that indicate a peripheral locus of action. Gander *et al.* (3) reported that when peritoneal exudate cells were incubated with 4 m*M* sodium salicylate, the pyretic effect of the supernatant fluid containing the leukocytic pyrogen was significantly reduced. Further, in rabbits treated with sodium salicylate, the fever caused by endotoxin was reduced, but that caused by leukocytic pyrogen was not. Since the fever induced by endotoxin is believed to result from release of leukocytic pyrogen, Gander and co-workers suggested that the antipyretic effect of sodium salicylate is due to peripheral action on the leukocytes which prevents formation or release of pyrogen.

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The present investigation was an attempt to determine whether sodium acetylsalicylate inhibits the release of the endogenous pyrogen from the leukocytes. The results clearly indicate that it does not have such an action.

Methods. White albino rabbits of both sexes, weighing 2.5 to 3.0 kg, were used. Animals with rectal temperatures higher than 39.6° were excluded from the experiment. Environmental temperature was maintained at 22 ± 0.5° throughout the course of study. Rectal temperature was monitored by means of a Tri-R, Model TP-6, flexible rectal thermistor probe and was recorded continuously on a Grass 5B polygraph.

A. Preparation of pyrogen. All glassware and instruments were sterilized in an autoclave for 30 min. Pyrogen was prepared according to the technique described by Rafter *et al.* (4) with minor modifications. In rabbits anesthetized with sodium pentobarbital, polymorphonuclear leukocytes were obtained from the peritoneal exudates 14–16 hr after intraperitoneal injection of 500 ml of 0.15 *M* NaCl, containing 1 g of shellfish glycogen, 20,000 units of penicillin G, and 0.25 g of streptomycin sulfate. The sterile exudates from each rabbit were pooled in an iced flask containing 1000 units of heparin. The pooled exudates were centrifuged at 275*g* for 20 min at 4°. The supernatant was decanted and the cells were resuspended in pyrogen-free saline solution (0.15 *M*) buffered with potassium phosphate at pH 7.10 to make a final suspension of 1×10^8 leukocytes/ml. The cell suspension was incubated in a water bath at 37° for 2 hr with continuous gentle shaking. Subsequently, the incubated suspen-

TABLE I. Effects of Sodium Acetylsalicylate on the Release of Leukocytic Pyrogen from Rabbit Peritoneal Exudate Cells *in Vitro*.

Sodium acetylsalicylate (m*M*)	No. of animals	Fever index in rabbits (2 hr)		
		Mean	SD	*p* values
0	6	15.63	2.32	
2	6	16.36	4.09	<0.3
4	6	16.75	4.55	<0.3
6	6	13.28	5.18	<0.1
10	6	16.99	2.50	<0.1
20	6	17.17	2.19	<0.1

sion was centrifuged at 4° and the supernatant, which contained the crude leukocytic pyrogen, was dialyzed against physiological saline solution to remove the potassium phosphate and stored at 4°.

The effects of sodium acetylsalicylate on the release of pyrogen from leukocytes *in vitro* were studied according to the method of Gander *et al.* (3), except for minor modifications. Sodium acetylsalicylate (4.04 to 40.4 mg) was added into 10 ml of phosphate buffered saline containing 1×10^8 leukocytes/ml, adjusted to pH 7.0–7.2, to make 2 to 20 m*M* solutions. The cells were incubated for 2 hr at 37° and were removed by centrifugation. The supernatant was dialyzed against physiological saline solution for a period of 72 hr to remove the residual sodium acetylsalicylate and phosphate buffer. A small amount of sodium ^{14}C-acetylsalicylate was added to ascertain whether or not dialysis was complete. The fluid containing leukocytes, incubated and treated in the same manner without sodium acetylsalicylate, served as control.

B. Assay for pyrogenicity. One milliliter of the supernatant fluid was administered to each rabbit via the marginal ear vein for determination of pyrogenicity. The resulting febrile responses were observed for 2 hr. The intensity of the fever was expressed by an arbitrary fever index obtained by measuring the area under the 2-hr fever curve (5).

Results. I. Incubation of exudative cells with sodium acetylsalicylate. The febrile reaction elicited by the supernatant fluid obtained from the incubate of peritoneal exudate cells with amounts of sodium acetylsalicylate ranging from 2 to 20 m*M* was determined in six rabbits with time intervals of 5–6 days between each injection.[2] Table I shows that such supernatant fluid produced fever with average fever indices varying from 13 to 17, which are not significantly different from those of the control groups of rabbits.

II. Dialysis of supernatant fluid containing leukocytic pyrogen. The supernatant fluid obtained from incubation of the exudate cells with sodium acetylsalicylate was dialyzed against physiological saline for removal of the sodium acetylsalicylate and phosphate buffer. This dialyzed fluid was injected into five rabbits and the average fever index was 17.1. In a control study, the nondialyzed supernatant fluid produced fever in the same animals; the fever index was 14.6 (Table II). Furthermore, supernatant fluid obtained from the incubation of exudate cells alone produced fever of similar intensity, whether or not it had been dialyzed. The average fever index produced by the dialyzed supernatant was 19.7, while the undialyzed supernatant produced an average fever index of 16.1 (Table II). Again, the difference is not significant.

III. Incubation of leukocytic pyrogen with sodium acetylsalicylate. The supernatant fluid containing leukocytic pyrogen, which was derived from incubation of the exudate cells, was incubated with 4 m*M* sodium acetylsalicylate in a water bath at 37° for 18 hr. This fluid produced fever in five rabbits with an average fever index of 14.0 (Table III). Control experiments, in which no sodium acetylsalicylate was added to the cells, yielded supernatants that produced fever with an average fever index of 13.6 in five other rabbits.

Discussion. The findings of the present investigation show that the presence of sodium acetylsalicylate during incubation of the peri-

[2] It was shown by King and Wood, Jr. (6), that rabbits do not exhibit tolerance to leukocytic pyrogen and therefore can be used repeatedly after rest periods of several days.

TABLE II. Effects of Sodium Acetylsalicylate and Phosphate Buffer on the Pyretic Effect of Leukocytic Pyrogen.

Peritoneal exudate cells in phosphate buffer	Dialysis[a]	Fever index in rabbits (2 hr)			
		No. of animals	Mean	SD	p values
Incubated with 4 mM sodium acetylsalicylate	No	5	14.59	2.35	<.1
	Yes	5	17.09	2.47	
Incubated, no sodium acetylsalicylate added	No	5	16.08	4.92	<.1
	Yes	5	19.66	2.44	

[a] Dialysis was done against 0.15 M physiological saline solution for 72 hr.

toneal exudate cells did not affect the release of pyrogen from the cells. The presence of a small amount of acetylsalicylate and phosphate buffer in the supernatant fluid did not affect the pyretic effect when the supernatant was injected into rabbits. The leukocytic pyrogen of the cells was also not destroyed after incubation of the pyrogen for 18 hr in the presence of sodium acetylsalicylate.

These findings are, therefore, at variance with those reported by Gander *et al.* (3). It is difficult to explain why the two sets of results were completely different. The experimental methods were similar in nature although there were minor technical differences. Gander *et al.* used sodium salicylate in the incubation with leukocytes and the maximum amount of salicylate was 4 mM. In the present experiments sodium acetylsalicylate was used and the amount varied from 2 to 20 mM. It is remotely possible that salicylate and acetylsalicylate do not act similarly. Gander *et al.* incubated the exudate cells overnight (18 hr), while in our experiments incubation lasted for 2 hr. It should be noted that incubation of the exudate cells for 2 hr has been reported to be sufficient for maximal release of pyrogen from the cells (7). Therefore, incubation of 2-hr duration should be sufficient to allow acetylsalicylate to act on the white blood cells. In an earlier report (2), we showed that the antipyretic action of sodium acetylsalicylate became evident in about 30 min after intravenous administration. Indeed, it was very effective in reducing the febrile reaction by leukocytic pyrogen in the monkey. Pretreatment of animals with sodium acetylsalicylate was also found effective in increasing the latency and decreasing the intensity of fever induced by leukocytic pyrogen (8). These findings also contradict those of Gander *et al.* (3), and lead us to conclude that the locus of antipyretic action of sodium acetylsalicylate is not peripheral, acting on the circulating leukocytes, but in the central nervous system.

It is well documented that an important temperature regulating mechanism for heat loss exists in the preoptic/anterior hypothalamic region (9). Recently various investigators have successfully recorded increased activity of single thermosensitive neurons in this area in response to local heating (10, 11) or to increased ambient temperature (12). This increased neuronal activity was depressed by typhoid vaccine (13) or bacterial pyrogen (1). Subsequent administration of sodium acetylsalicylate resulted in a return of thermal responsiveness. Furthermore, it was observed that sodium acetylsalicylate is more effective when injected into the carotid artery than intravenously (1). These results clearly indicate that sodium acetylsalicylate acts, perhaps competitively with pyrogen, at some specific receptor site in the preoptic/anterior hypothalamic region of the central nervous system.

Summary. The effects of sodium acetylsalicylate on the release of pyrogen from peritoneal exudate cells were studied *in vitro* by incubation of the cells in solutions of sodium acetylsalicylate that varied in amounts from 2 to 20 mM. The ability of the leukocytes to release pyrogen, as reflected by the fever index of rabbits, was not altered by sodium acetylsalicylate. The antipyretic action of so-

TABLE III. Effects of Incubation of Leukocytic Pyrogen with Sodium Acetylsalicylate *in Vitro*.

Treatment of cells	No. of animals	Fever index (mean)	SD	*p* values
Control (with no acetylsalicylate) leukocytic pyrogen incubated	5	14.01	2.54	
Experimental	5	13.63	2.44	<.3

dium acetylsalicylate is not due to inhibition of release of leukocytic pyrogen. Our accumulated evidence indicates that acetylsalicylate has a central locus of antipyretic action, most likely in the preoptic/anterior hypothalamus.

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