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Influence of the duration of experimental fever on salicylate antipyresis in the rabbit

W. I. CRANSTON, R. H. LUFF, M. D. RAWLINS AND V. ANN WRIGHT

Department of Medicine, St. Thomas's Hospital Medical School, London SE1

Summary

1. Steady state fever has been produced in rabbits with a priming injection followed by a sustaining infusion of homologous plasma containing endogenous pyrogen (EP). This fever appears to last as long as the infusion continues.

2. Intravenous salicylate given 1 h after the start of the EP infusion produced only a small antipyretic effect. The same dose of salicylate given 4 h after the start of an EP infusion resulted in rapid and progressive defervescence. Intermediate antipyretic responses were obtained when salicylate was administered intravenously 2 and 3 h after the start of an EP infusion.

3. Less than 1% of the systemic dose, when injected into a lateral cerebral ventricle, produced a significantly smaller response at 1 h than at 4 h after the start of an EP infusion. At both these times the fall in temperature following the intraventricular salicylate injection was dose dependent, but the slope of the dose-response line was significantly steeper at 4 h than at 1 hour.

4. It is suggested that salicylates produce their antipyretic effects by antagonizing the action of EP within the nervous system, and that the hypothalamic EP concentration falls during the course of an EP infusion.

Introduction

Recent studies on the mechanism of the antipyretic properties of salicylates have led to conflicting conclusions. Gander, Chaffee & Goodale (1967) found that treatment of rabbits with intravenous salicylate reduced the subsequent febrile response to intravenous bacterial pyrogen but not to endogenous pyrogen (EP). They furthermore produced evidence that, *in vitro,* salicylates reduced the yield of EP from rabbit leucocytes incubated with bacterial pyrogen, and suggested that the sole mechanism of salicylate induced antipyresis was by the suppression of EP release from cells. Grundman (1969) was also unable to demonstrate a diminished febrile response to intravenous EP in rabbits treated with salicylates in a small series of experiments, but could do so with a large series using a crossover technique (Cooper, Grundman & Honour, 1968). These workers found, however, that pretreatment with intravenous salicylate did not suppress the febrile response to EP injected into the rabbit cerebral ventricle and suggested that salicylates might act by interfering with the passage of EP into the hypothalamus and pre-optic areas of the brain.

In both man (Adler, Rawlins, Rosendorff & Cranston, 1969) and the rabbit (Cranston, Luff, Rawlins & Rosendorff, 1970a) it is possible to produce a steady

state fever by an intravenous priming injection followed by a sustaining infusion of EP. In man, 2 g of sodium salicylate given intravenously 2·5 h after the EP infusion produced rapid and progressive defervescence while the EP infusion continued. In the rabbit, both intravenous and intraventricular salicylate (the latter dose representing 1% of the former) induced a dose dependent fall in temperature when given 4 h after the start of an EP infusion. These results suggest that salicylates do not exert their antipyretic effect solely by interfering with formation or release of EP from cells and that at least part of this additional mechanism is mediated via a direct action of salicylate within the central nervous system.

Both intravenous and intraventricular salicylate produce antipyresis when administered to rabbits during an established fever induced by intraventricular EP (Cranston, Hellon, Luff, Rawlins, & Rosendorff, 1970b). This is incompatible with the hypothesis of Cooper *et al.* (1968) that salicylates produce their central antipyretic effect by preventing the passage of EP into the anterior hypothalamus and pre-optic areas of the brain.

There is thus a considerable conflict of views on the way in which salicylates act, and this appears to derive from differences in experimental design. In general, it has been found more difficult to influence pyrogen induced fever by pretreatment with salicylate than by treatment during an established fever. We have attempted to study this by observing the antipyretic responses induced by both intravenous and intraventricular salicylate at various times after the start of an EP infusion.

Methods

Endogenous pyrogen was prepared as described previously (Cranston *et al.*, 1970a) by incubating rabbit whole blood with purified *Proteus* endotoxin ('E' pyrogen, Organon Laboratories) at a concentration of 0·003 μg/ml for 18 h at 37° C. The plasma was separated by centrifugation at 2,000 *g* for 30 min and stored at +4° C until used.

In all experiments the animals were restrained in conventional stocks and their rectal temperatures measured with thermistors inserted 5–10 cm. The thermistors were attached to a ten-channel United Systems Corporation (Digitec) thermometer and each animal's rectal temperature was printed out every 5 minutes. A size 00 nylon catheter (Portex) was advanced 4–8 cm into a marginal ear vein and when the animal's rectal temperature had stabilized, fever was induced by a priming injection of 2·5 ml homologous plasma containing EP followed by a sustaining infusion of 0·02 ml/minute.

In the first group of experiments using New Zealand white rabbits of both sexes and weighing 2–3 kg, a second 00 nylon catheter was inserted into the marginal ear vein of the other ear and kept patent with a slow infusion of 0·9% sodium chloride. This was used subsequently for the administration of 240 mg sodium salicylate in 20 ml 0·9% sodium chloride, followed by a sustaining infusion of salicylate at a rate of 1·5 mg/minute. The salicylate was given at 1, 2, 3 or 4 h after the start of the EP infusion and the animals' rectal temperatures were observed for 80 min thereafter. Six animals were used in each series and at the end of every experiment blood was withdrawn from an ear artery into a heparinized syringe for the estimation of total plasma salicylate concentration by a modification of the method of Udenfriend (1962). In a further series of experiments using eleven animals, no

salicylate was given after the start of the EP infusion which was continued for 5·5 h, and rectal temperature was measured continuously ; these acted as controls.

In the second group of experiments modified Monnier-Gangloff headplates (Cooper, Cranston & Honour, 1967) were attached to skulls of chinchilla rabbits of both sexes weighing 2·5–3·5 kg under general anaesthesia. At least a week was allowed to elapse for the animals to recover. At the start of each experiment a stainless steel cannula attached to a syringe by about 20 cm, 00 nylon tubing and filled with artificial cerebro-spinal fluid (C.S.F.) (Cameron & Semple, 1968) was inserted into one lateral cerebral ventricle using the co-ordinates of Monnier & Gangloff (1961). In all the experiments fever was produced as described above by an intravenous priming injection followed by a sustaining infusion of homologous rabbit EP. Two separate series of rabbits were used. The first, consisting of seven animals, received on different days, an intraventricular injection of 1·2 mg and 0·6 mg sodium salicylate in 0·4 ml artificial C.S.F. and a control injection of 0·4 ml artificial C.S.F. alone 1 h after the start of the EP infusion. Each animal therefore received two doses of salicylate and acted as its own control. At least 48 h were allowed to elapse between intraventricular injections in any one animal. The same dose schedule was given to the second series of five animals 4 h after the start of the EP infusion, and again, each animal acted as its own control. Rectal temperature was observed for 80 min after the intraventricular injection.

Results

Intravenous salicylate

As can be seen in Fig. 1, a priming injection of 2·5 ml homologous plasma containing EP followed by a sustaining infusion of 0·02 ml/min produced a rapid rise in temperature of 0·8–1·2° C over the first 60 min in the control series of eleven rabbits. Thereafter, the temperature remained reasonably stable and the steady state fever persisted for the duration of the EP infusion. An intravenous injection of 240 mg sodium salicylate followed by a sustaining infusion of 1·5 mg/min given 4 h after the start of the EP infusion produced a progressive fall in temperature over the subsequent 80 min, and in these animals a mean fall in rectal temperature of 0·72° C was observed at this time (S.E. ±0·18) (Fig. 2). However, it can be seen

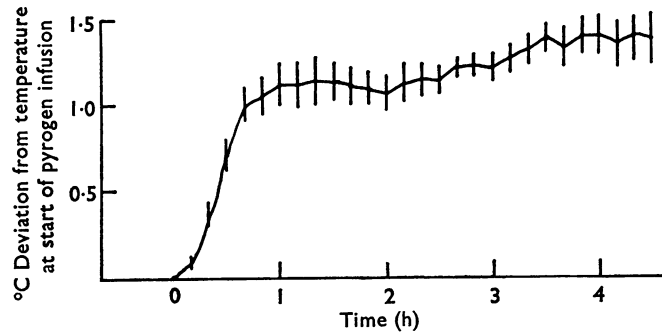

FIG. 1. Mean rectal temperature responses of eleven rabbits to a 2·5 ml priming injection followed by a sustaining infusion of 0·02 ml/min of homologous plasma containing endogenous pyrogen. Ordinate: deviation (° C) from temperature at start of infusion. Abscissa: time in hours after the start of the infusion. Vertical lines represent ±1 standard error.

from this figure that the same dose of salicylate given 1 h after the start of the EP infusion produced little change in rectal temperature and that salicylate given at 2 and 3 h produced intermediate responses. There is a significant correlation between the fall in temperature at 80 min following 240 mg salicylate and the time after the start of the EP infusion at which it was given ($r = -0{\cdot}50$ $P < 0{\cdot}02$).

It is clear from Table 1 that the group receiving salicylate 1 h after the start of the EP infusion had a significantly higher mean total plasma salicylate concentration than the other groups. However, this group showed the least change in rectal temperature following the administration of salicylate. The other three groups had similar total plasma salicylate concentrations, and therefore the differences in the antipyretic responses to salicylates between the groups cannot be attributed to differences in plasma salicylate concentration.

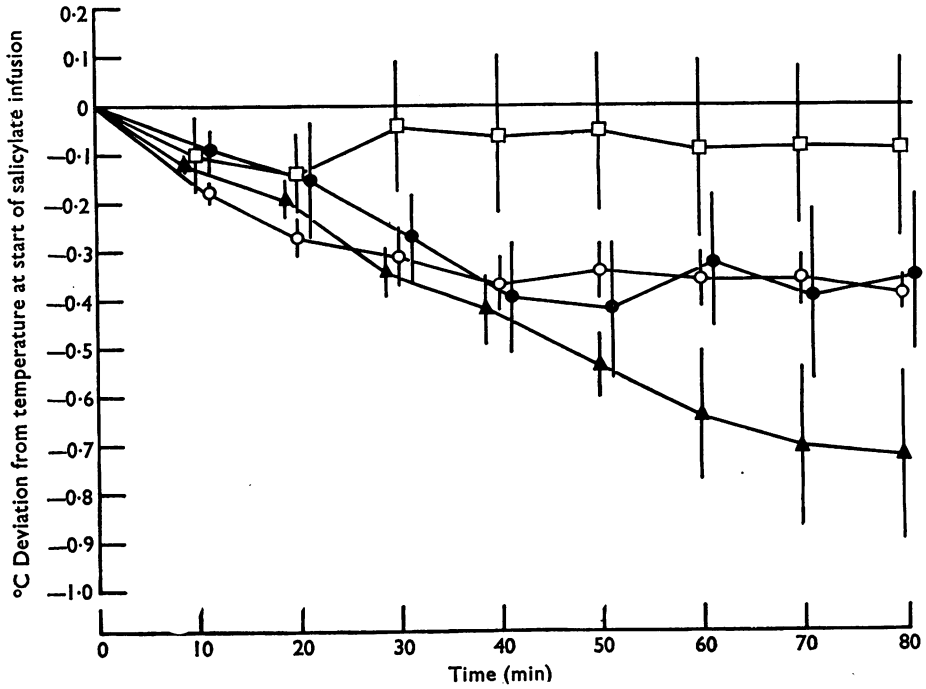

FIG. 2. Mean rectal temperature responses of four groups of rabbits to 240 mg sodium salicylate (intravenous) followed by an infusion at 1·5 mg/min given at either 1, 2, 3 or 4 h after the start of an infusion of endogenous pyrogen. Each group contained six rabbits. Ordinate: deviation (° C) from the temperature at the time the salicylate was given. Abscissa: time (min) after the salicylate was given. Vertical lines represent ±S.E. □, 1 h; ●, 2 h; ○, 3 h; ▲, 4 hours.

TABLE 1. *Plasma salicylate concentrations in animals given intravenous salicylate infusions at different times after E.P. injections*

Time after start of E.P. infusion (h)	No. of animals	Plasma salicylate (mg/100 ml) Mean±S.D.
1	6	36·9±4·1
2	6	27·5±4·6
3	6	31·6±1·4
4	6	30·6±3·4

Intraventricular salicylate

Figure 3 shows the changes in rectal temperature following the intraventricular injection of 0·6 mg and 1·2 mg sodium salicylate. For each animal the temperature after control C.S.F. injections was subtracted from the temperature observed at the same time after salicylate injection, to give the change of temperature attributable to the salicylate. In Fig. 3 responses are shown when intraventricular injections were given 1 h and 4 h after the start of the E.P. infusion.

In both series there is clearly a dose dependent fall in temperature following the salicylate administration which reaches a nadir 30–40 min after the injection and which subsequently either shows little change or returns towards the control temperature. In Fig. 4, it can be seen that there is a clear relationship between the change in temperature 40 min after the intraventricular injection, and the dose of salicylate administered at both 1 and 4 h after the start of the EP infusion (correlation coefficients $r=-0{\cdot}48$, $P<0{\cdot}05$; $r=-0{\cdot}86$, $P<0{\cdot}001$, respectively). However, the slope of the dose-response regression line 4 h after the start of the EP infusion ($-0{\cdot}72°$ C mg^{-1}) is significantly different from the slope of the regression line ($-0{\cdot}25°$ C mg^{-1}) from the 1 h series ($t=3{\cdot}12$; $P<0{\cdot}001$).

Discussion

It is clear from the results of those experiments in which intravenous salicylate was given at varying times after the start of the EP infusion, that the magnitude of the salicylate induced antipyresis increases with the duration of the fever. Thus, intravenous salicylate given at 1 h after the start of the EP infusion has little antipyretic effect whilst at 4 h it produces rapid defervescence. This is compatible with

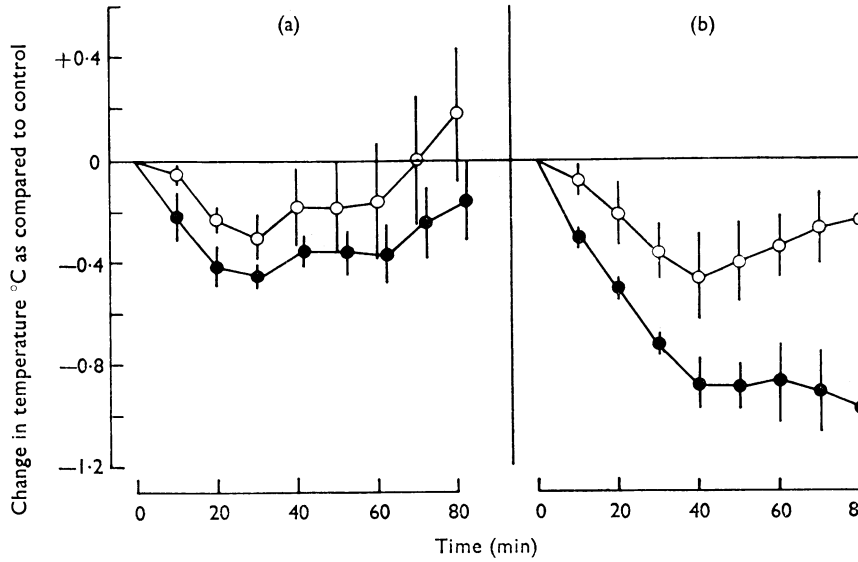

FIG. 3. Mean changes in rectal temperature following the administration of 0·6 mg (○) and 1·2 mg (●) of sodium salicylate into a lateral cerebral ventricle. (a) Salicylate given 1 h after the start of an endogenous pyrogen (EP) infusion. (b) Salicylate given 4 h after the start of an EP infusion. Ordinate: deviation (° C) from temperature at time of intraventricular injection as compared to control (receiving 0·4 ml artificial cerebro-spinal fluid); Abscissa: time in minutes following salicylate injection. Vertical lines represent ±1 S.E.M.

the observations made in animals that pre-treatment with salicylate produces little alteration in the febrile response to a single intravenous injection of EP. The fact that the antipyretic response to intraventricular salicylate is similarly related to the duration of the EP infusion suggests that the increasing antipyretic response to intravenous salicylate during the EP infusion is not due to changes in permeability of the blood-brain barrier to salicylate, but this possibility cannot be regarded as completely disproved.

Salicylates have no effect upon either the temperature or the thermoregulatory reflexes of afebrile human subjects (Rosendorff & Cranston, 1968), nor on the temperature of afebrile rabbits (Cranston *et al.*, 1970a). In addition, salicylates do not produce antipyresis when given to rabbits with fever induced by local cooling of

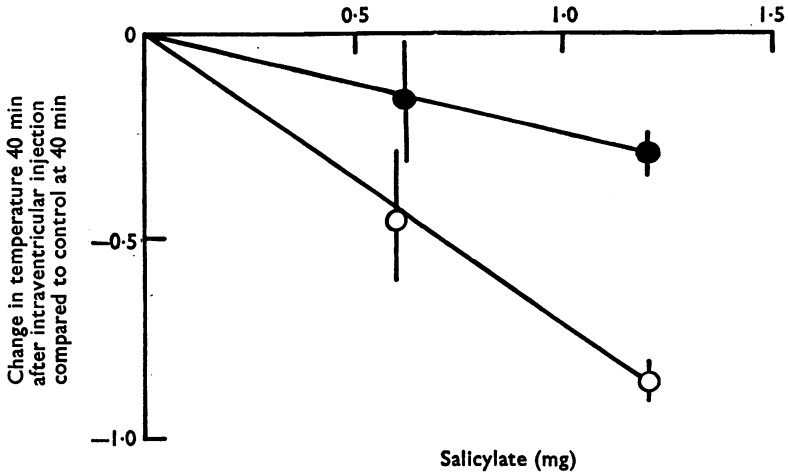

FIG. 4. Dose-response relationship between dose of intraventricular salicylate and the fall in temperature which it induces at 1 h (●) and 4 h (○) after the start of an endogenous pyrogen infusion. Ordinate: change in rectal temperature (° C) 40 min after intraventricular injection compared with control at 40 minutes. Abscissa: intraventricular salicylate dose (mg). Vertical lines represent ±1 S.E.M. The regression lines are also included.

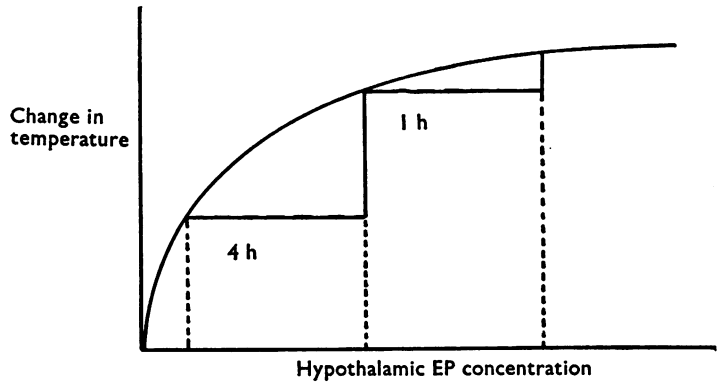

FIG. 5. Hypothetical relationship between the hypothalamic endogenous pyrogen (EP) concentration and the change in body temperature it induces. It is suggested that salicylates might act by reducing the effective hypothalamic EP concentration by competitive antagonism. At a high hypothalamic EP concentration (1 h after the start of an intravenous EP infusion) salicylates would exert little effect on temperature. At a lower hypothalamic EP concentration (4 h after the start of an intravenous EP infusion) the effect would be much greater.

the hypothalamus (Cranston *et al.*, 1970b). It is reasonable to hypothesize therefore, that salicylates exert their antipyretic effect by antagonizing either directly or indirectly the action of EP upon the central nervous system.

In order to explain the results of the experiments described in this paper it is necessary to propose three hypotheses. (1) Salicylates produce their antipyretic effects by antagonizing the action of EP upon the central nervous system competitively. (2) The relationship between the hypothalamic concentration of EP and the induced temperature change is non-linear (Fig. 5). (3) Following a priming injection followed by a 4 h sustaining infusion of EP there is an early rise in the hypothalamic concentration of EP, due to the priming injection, which reaches a maximum at 30–90 minutes. Following this, there is a gradual fall in the hypothalamic EP concentration with little change in rectal temperature.

There is indirect evidence in support of all of these hypotheses. In the first place, it can be seen from Fig. 3 that intraventricular injection of salicylate produces a transient fall in temperature; the later rise has been attributed to clearance of salicylate from the central nervous system (Cranston *et al.*, 1970a). If salicylates acted non-competitively a progressive fall in temperature, qualitatively similar to that produced by intravenous salicylates, would be expected. Second, Murphy (1966) has shown that there is a linear logarithmic relationship between the dose of intravenous EP and the febrile response, and it is reasonable to suppose that a similar relationship holds for hypothalamic EP concentration and the febrile response. It is likely, however, that other factors are involved in the genesis of the very high biphasic fevers seen in rabbits given very large doses of either endotoxin or endogenous pyrogen intravenously (Bornstein, Bredenberg & Wood, 1963). Third, it has been shown that the concentration of circulating EP 4 h after the start of an EP infusion is only 50% of the concentration at 1 h (Rawlins, Rosendorff & Cranston, 1970), even though there is no significant fall in rectal temperature during this period. It is possible, therefore, that a similar fall in the hypothalamic EP concentration might occur during a 4 h EP infusion.

On the basis of these hypotheses the increasing magnitude of the antipyretic response to intravenous salicylate during an EP infusion might be due to the action of this drug in competing with the effects of EP within the hypothalamus. Due to the non-linearity of the relationship between hypothalamic EP concentration and the temperature change, salicylates would be less effective at 1 h after the start of an EP infusion (when the hypothalamic EP concentration is higher) than at 4 h (Fig. 5). The fact that the slope of the 1 h dose-response relationship with intraventricular salicylate is significantly less than the 4 h dose-response relationship, is compatible with this hypothesis.

The inability of various workers, therefore, to show that pretreatment of rabbits with salicylate depresses the febrile response to intravenous or intraventricular EP injections could be due to the fact that these injections cause an early high concentration of EP within the nervous system so that salicylates are relatively ineffective.

In the present experiments it is assumed that the passage of time permits hypothalamic concentration of EP to fall considerably, but that the temperature rise is unaffected because of the shape of the curve relating hypothalamic EP concentration to temperature change. In these circumstances, as in naturally occurring fever, the antipyretic action of salicylate can be demonstrated. If this explanation is true, it may be difficult to assess the antipyretic activity of different drugs, by giving them

to test animals and observing the temperature response to subsequent intravenous injections of EP.

Pretreatment of animals with salicylate appears to be much more effective in the prevention of febrile responses to injections of bacterial pyrogen (Gander *et al.*, 1967). In this situation the peripheral mechanism described by Gander *et al.* (1967) may be acting as well as a central mechanism.

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