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EFFECTS OF INTRACEREBRAL MICRO-INJECTION OF SODIUM SALICYLATE ON TEMPERATURE REGULATION IN THE RABBIT

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SUMMARY

1. Symmetrical, bilateral micro-injections (10 μl.) of 6–30 μg sodium salicylate have been made into various parts of the brains of febrile and afebrile rabbits.

2. In rabbits with fever induced by an intravenous infusion of endogenous pyrogen, micro-injections of sodium salicylate produced antipyresis when given into the preoptic hypothalamus and the mid-brain.

3. In afebrile rabbits, micro-injections of sodium salicylate into these areas were without effect on temperature.

4. The results suggest that at least part of the antipyretic effect of salicylates may be mediated by antagonizing the effects of endogenous pyrogen within these areas of the brain.

INTRODUCTION

Recent evidence suggests that salicylates produce their antipyretic effects in two ways. In the first place, Gander, Chaffee & Goodale (1967) obtained evidence to suggest that salicylates reduced the yield of endogenous pyrogen from white blood cells: however, the fact that in man intravenous salicylate produces defervescence during fever induced by an intravenous infusion of endogenous pyrogen, even when the pyrogen infusion is continued, suggests that this is not the sole mechanism (Adler, Rawlins, Rosendorff & Cranston, 1969). There is also evidence to suggest that salicylates might exert at least part of their action within the central nervous system since injections of small doses of sodium salicylate into the lateral cerebral ventricles of rabbits with experimental fever cause rapid, dose-dependent defervescence (Cranston, Luff, Rawlins & Rosendorff, 1970). Further evidence suggests that salicylates exert their central effect on temperature by competitively antagonizing the effects of endogenous

pyrogen within the brain (Cranston, Luff, Rawlins & Wright, 1971). The nature of this antagonism is not known, but if salicylates exerted their effects by antagonizing endogenous pyrogen directly, they might be expected to act at the same sites within the central nervous system as endogenous pyrogen. The work described in this paper was performed in order to locate the site of this central action of salicylate by injecting small quantities of salicylate into various areas of the brains of febrile and afebrile rabbits.

METHODS

Head-plates (Monnier & Gangloff, 1961) were attached to the skulls of chinchilla rabbits weighing 2·5–3·5 kg under pentobarbitone sodium anaesthesia (25–35 mg/kg). At least 1 week was allowed to elapse before any experiments were performed. All experiments were performed in a temperature controlled room at 18–21° C. The animals were restrained in conventional head stocks and their rectal temperatures measured with glass bead thermistors inserted 8–10 cm. The thermistors were connected to a Digitec (United Systems Corporation) thermometer and temperatures were printed out every minute. Stainless-steel injection cannulae were inserted bilaterally into preselected areas of the brain using the stereotaxic co-ordinates of Monnier & Gangloff (1961). The injection cannulae were attached to micro-syringes (Hamilton) by 12 cm 00 nylon (Portex) and were filled with artificial cerebrospinal fluid (Cameron & Semple, 1968).

In fifty-one animals, when rectal temperature had stabilized, fever was induced with an intravenous priming injection followed by a sustaining infusion of homologous endogenous pyrogen. Endogenous pyrogen was prepared by incubating rabbit blood at 37° C for 18 hr with Proteus endotoxin (E pyrogen, Organon Laboratories) at a concentration of 3·0 ng/ml. as described previously (Cranston *et al.* 1970). The plasma containing endogenous pyrogen was separated by centrifugation at 2500 *g* for 30 min and stored at +4° C. This plasma was administered through a polyethylene catheter advanced 3–4 cm into the marginal vein of one ear at a priming dose of 2·5 ml. followed by a sustaining infusion of 0·01 ml./min; this infusion was continued for the duration of the experiment. Four hours after the start of the endogenous pyrogen infusion, when the animals had developed steady-state fevers, symmetrical and bilateral injections of 10 μl. were made intracerebrally through the stainless-steel injection cannulae. The injections were made simultaneously on each side and at least 20 min were allowed to elapse between pairs of injections; each injection was completed in 30 sec. Most animals received one injection of artificial cerebrospinal fluid alone and two to four injections containing 6–30 μg sodium salicylate in artificial cerebrospinal fluid. Since the dead space of each injection cannula and catheter was approximately 100 μl. it was necessary to withdraw the cannulae between each pair of injections and refill them with artificial cerebrospinal fluid containing salicylate at a different concentration. At the end of each experiment, the animal was killed with an intravenous injection of pentobarbitone sodium 60 mg/kg. The intracerebral cannulae were withdrawn and primed with Indian ink (Reeves) and re-inserted into the brain. 10 μl. ink were injected through the cannulae, and the brain was removed and fixed in 10 % formalin for at least 10 days. After embedding in 3 % agar, sections were cut at 500 μm intervals to locate the injection site. Fig. 1 shows the distribution of Indian ink following bilateral injection of 10 μl. of this material into the mid-brain.

Seventeen afebrile rabbits were given local intracerebral injections of artificial cerebrospinal fluid alone and containing sodium salicylate. When the animal's rectal temperature had stabilized, three pairs of micro-injections were made into bilateral symmetrical areas of the brain. Each pair of injections were separated by an interval of at least 20 min. The injectate contained either no salicylate, 12 μg or 30 μg sodium salicylate in 10 μl. artificial cerebrospinal fluid. At the end of each experiment the animal was killed and the injection site was stained with Indian Ink and identified as described above.

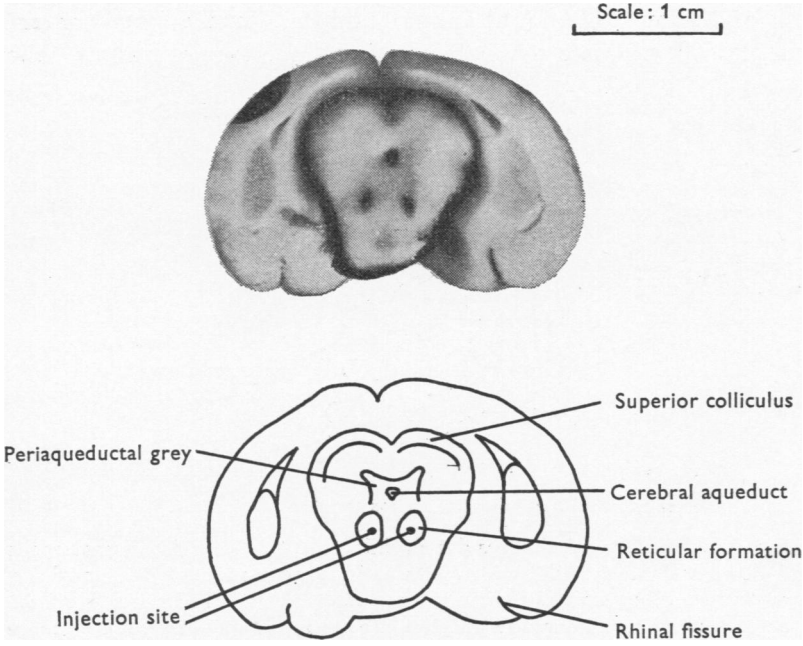

Fig. 1. Transverse section of the rabbit mid-brain at the level of the superior colliculus. This shows the distribution of 10 μl. Indian ink injected bilaterally.

After each experiment the animal's rectal temperature was plotted and the integral of the temperature response (in ° C min) following micro-injections was measured by planimetry. The intra-observer error was estimated by repeating the measurements 2 months later and comparing the results; this showed a reasonable correlation ($r = \pm 0{\cdot}926$; slope = 1·002; S.E. = $\pm 0{\cdot}070$). The mean of the pair of measurements thus obtained was used in the analysis of the results.

RESULTS

Febrile animals

Infusion of endogenous pyrogen resulted in rectal temperature rises of 0·5–2·0° C over the first 60 min, followed by sustained fever for as long as this pyrogen infusion continued.

The bilateral injection of 10 μl. artificial cerebrospinal fluid into febrile animals usually had no effect upon temperature. In five animals a small

and transient fall of temperature was observed; the greatest fall measured 2·38° C min. The injection of artificial cerebrospinal fluid containing 6–30 μg sodium salicylate was succeeded by either no change or a fall in rectal temperature. In eleven animals the temperature changes in response to bilateral microinjections of salicylate exceeded 2·38° C min. The injection sites where these responses were elicited (Fig. 2) were the preoptic region (five animals), the periaqueductal grey area of the mid-brain (four animals), the cerebellum (one animal) and the hippocampus (one animal). Examples of responses to bilateral micro-injections of sodium salicylate

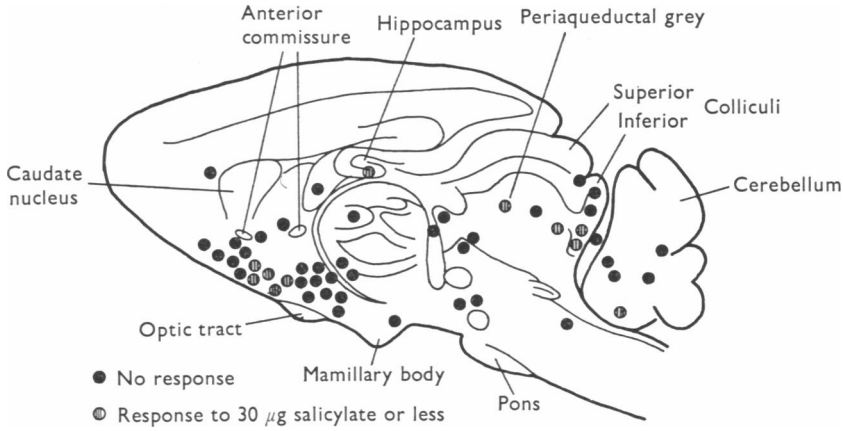

Fig. 2. Summary of the results of bilateral (10 μl.) micro-injections of 6–30 μg sodium salicylate in rabbits with pyrogen-induced fever projected on to a mid line sagittal section of the rabbit brain. Filled circles represent areas where micro-injections of salicylate produced no antipyretic response; hatched circles represent areas where significant antipyretic responses were produced by micro-injections of salicylate.

into the preoptic hypothalamus and the mid-brain are shown in Fig. 3. It can be seen in these two experiments that control injections had no effect upon temperature. Injection of 12 μg salicylate into the preoptic region caused a temperature fall of 0·4° C, followed by a gradual rise to control levels. A similar temperature fall was produced by an injection of 6 μg salicylate into the mid-brain. Fig. 4 demonstrates the absence of any change in temperature of an animal which received 6 μg and 12 μg salicylate by bilateral micro-injections into the posterior hypothalamus.

The mean rise in rectal temperature due to the pyrogen infusion was slightly higher in those animals which showed significant antipyretic responses (1·57 S.D. $\pm$ 0·66° C) as compared to those which were unresponsive (1·41 S.D. $\pm$ 0·49° C). The difference was not significant ($t = 0{\cdot}876$, $P > 0{\cdot}1$).

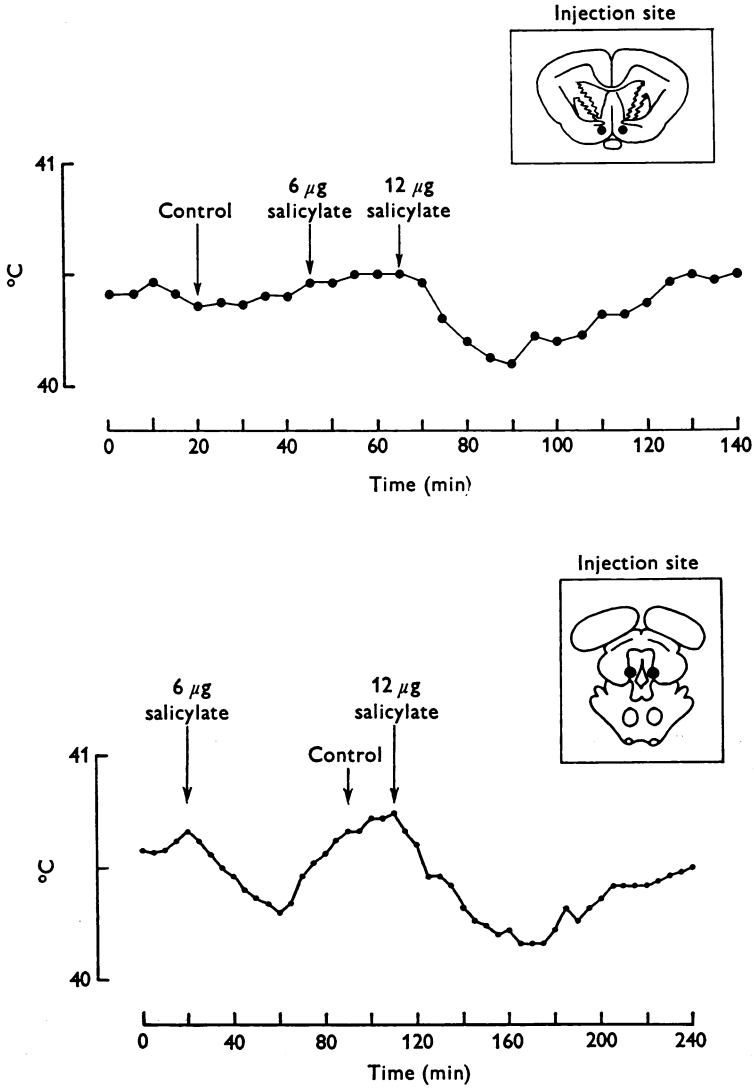

Fig. 3. The effect of bilateral (10 μl.) micro-injections of artificial cerebrospinal fluid alone and containing 6 μg and 12 μg sodium salicylate on rectal temperature in rabbits with pyrogen-induced fevers. The accompanying diagrams show the injection sites which were in the preoptic hypothalamus (upper trace) and the periaqueductal grey matter of the mid-brain (lower trace). Ordinates: rectal temperature (° C). Abscissae: time in minutes.

Afebrile animals

In this series of experiments, local intracerebral injections of salicylate were limited to those areas of the brain except the hippocamus where significant responses were produced by micro-injections of salicylate in

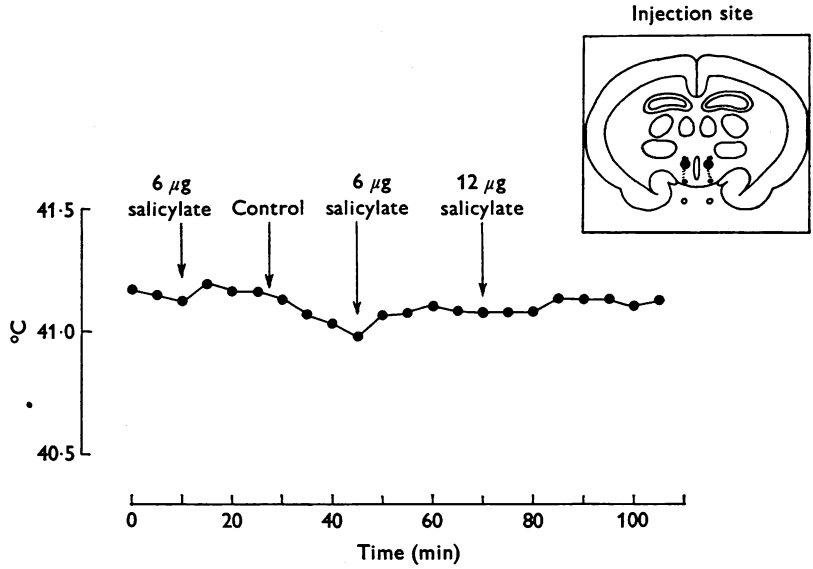

Fig. 4. The effect of bilateral (10 μl.) micro-injections of articial cerebrospinal fluid alone and containing 6 μg and 12 μg sodium salicylate on rectal temperature in a rabbit, with a pyrogen-induced fever. The injection site (the posterior hypothalamus) is shown in the accompanying diagram. Ordinate: rectal temperature (° C). Abscissa: time in minutes.

febrile animals (Fig. 5). None of the afebrile rabbits showed any significant change in temperature following the micro-injection of either artificial cerebrospinal fluid alone, or containing 12 μg or 30 μg sodium salicylate. The effects on rectal temperature of micro-injections of these doses of salicylate into the preoptic hypthalamus and mid-brain of afebrile rabbits are shown in Fig. 6.

DISCUSSION

It is clear from these results that some febrile animals responded to bilateral, symmetrically placed micro-injections of artificial cerebrospinal fluid with a small fall in temperature. Artificial cerebrospinal fluid was chosen for the control injections and as the vehicle for the salicylate injections because it approximated to brain extracellular fluid. Since no afebrile animal showed any change in temperature after control micro-

injections, the transient fall observed in five febrile animals was probably coincidental with the small fluctuations in temperature that occur in rabbits with fever. It is reasonable, therefore, to regard changes in temperature following the micro-injection of salicylate as representing significant antipyresis only if the response falls outside the range of responses to control injections. On this basis, antipyretic responses were grouped in two areas (Fig. 2), the preoptic hypothalamus and the periaqueductal grey area of the mid-brain. One febrile animal showed a fall of temperature following the injection of 6 μg salicylate into the hippocampal area. This

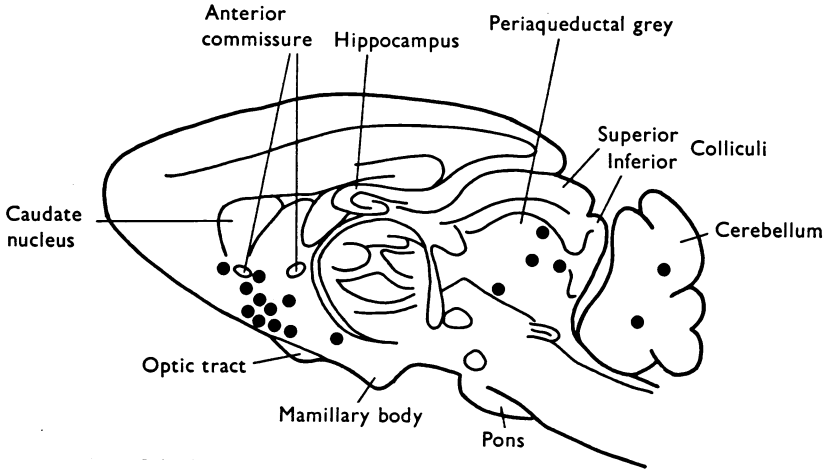

Fig. 5. Sites of bilateral (10 μl.) micro-injections of 12 μg and 30 μg sodium salicylate in afebrile rabbits projected on to a mid line coronal section of the brain.

animal showed no response to injections of 12, 18 or 30 μg, and it seems likely that this response may have been an artifact. When responses were obtained in the preoptic region or mid-brain, these did not show an inverse dose effect relationship of this kind. In another animal, a response greater than the maximum fall after control injections was seen following injection into the ventral part of the cerebellum. No other cerebellar injections caused antipyresis, but there was no evidence, from Indian ink injection, that the injected fluid had tracked into the adjacent mid-brain, which might have been a possible explanation of this observation.

Although relatively large volumes (10 μl.) of fluid were injected intracerebrally in these experiments only a few of the febrile animals (and none of the afebrile rabbits) developed any change in temperature in response to artificial cerebrospinal fluid alone. In addition Rosendorff (1969) has shown that repeated injections of up to 10 μl. fluid into the preoptic hypothalamus

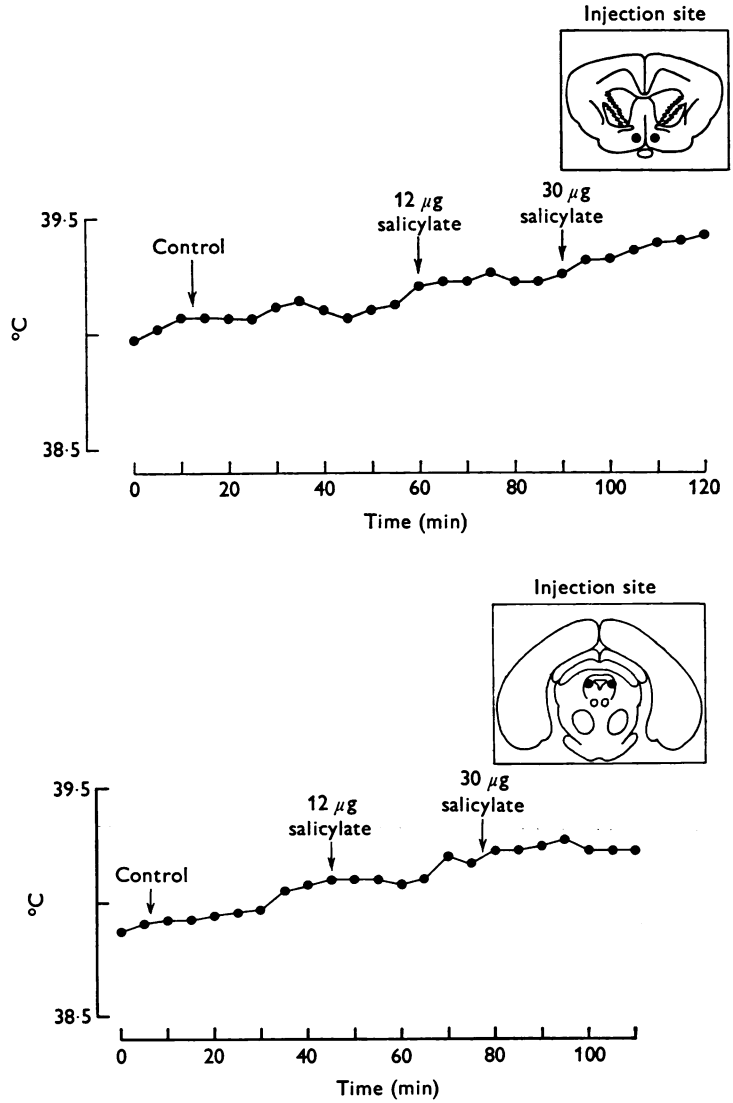

Fig. 6. The effect of bilateral (10 μl.) micro-injections of artificial cerebrospinal fluid alone and containing 12 μg and 30 μg sodium salicylate on rectal temperature in afebrile rabbits. The accompanying diagrams show the injection sites which were in the preoptic hypothalamus (upper trace) and the periaqueductal grey matter of the mid-brain (lower trace) Ordinates: rectal temperature (° C). Abscissae: time in minutes.

has no effect on either rectal temperature or autoregulation of local cerebral blood flow in the rabbit. The possibility of some of the injectate flooding into the ventricular cavity cannot be excluded, but the antipyresis observed with 30 μg salicylate or less intracerebrally was considerably greater than that observed in animals receiving 120 μg salicylate intraventricularly (Cranston *et al.* 1970). It appears from these experiments, therefore, that in febrile rabbits local bilateral micro-injections of 6–30 μg salicylate produce antipyresis in two areas of the brain, viz. the preoptic hypothalamus and the mid-brain. The responses are qualitatively similar to those observed after intraventricular injections of salicylate (Cranston *et al.* 1970) with temperature falling to a nadir 20–40 min after intracerebral injection followed by a rise towards pre-injection levels thereafter. The volume of distribution of injected salicylate is not known, so that its effective concentration cannot be calculated and related to levels found after systemic administration. It is in any event likely that the concentration changes quite rapidly due to local diffusion into the cerebrospinal fluid and clearance by blood flow. The transient nature of the temperature responses would support this. The effective antipyretic doses administered intracerebrally were only about 10% of the intraventricular doses. In afebrile rabbits, local injections of salicylate into the preoptic hypothalamus and the periaqueductal grey area of the mid-brain had no effect upon temperature and this, too, is similar to the response of afebrile rabbits to intraventicular salicylate (Cranston *et al.* 1970). If the antipyretic action of salicylate is mediated in the same manner, whether the drug is given intravenously, intraventricularly or intracerebrally, this action may be exerted within the preoptic hypothalamus and the periaqueductal grey area of the mid-brain.

The fact that both these areas are involved is not unexpected. Both the preoptic hypothalamus (Cooper, Cranston & Honour, 1967; Jackson, 1967) and the mid-brain (Rosendorff, Mooney & Long, 1970) have been shown to respond to local injections of endogenous pyrogen by producing fever. Furthermore, both areas contain thermosensitive neurones (Hellon, 1967; Nakayama & Hardy, 1969) and whilst the preoptic hypothalamus contains high concentrations of monoamines thought to be involved in thermoregulation (Feldberg, 1968), the mid-brain contains cells bodies whose axons both reach the hypothalamus and are rich in 5-hydroxytryptamine (Dahlstrom & Fuxe, 1964). The observations are therefore compatible with the hypothesis (Cranston *et al.* 1971) that salicylates exert their antipyretic effect by antagonizing endogenous pyrogen within the central nervous system. They do not show, however, the mechanism by which this antagonism occurs.

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REFERENCES

ADLER, R. D., RAWLINS, M. D., ROSENDORFF, C. & CRANSTON, W. I. (1969). The effect of salicylate on pyrogen-induced fever in man. *Clin. Sci.* **37**, 91–97.

CAMERON, I. R. & SEMPLE, S. G. J. (1968). The central respiratory stimulant action of salicylates. *Clin. Sci.* **35**, 391–401.

COOPER, K. E., CRANSTON, W. I. & HONOUR, A. J. (1967). Observations on the site and mode of action of pyrogens in the rabbit brain. *J. Physiol.* **191**, 325–337.

CRANSTON, W. I., LUFF, R. H., RAWLINS, M. D. & ROSENDORFF, C. (1970). The effects of salicylate on temperature regulation in the rabbit. *J. Physiol.* **208**, 251–259.

CRANSTON, W. I., LUFF, R. H., RAWLINS, M. D. & WRIGHT, V. A. (1971). The influence of the duration of experimental fever on salicylate antipyresis in the rabbit. *Br. J. Pharmac.* **41**, 344–351.

DAHLSTROM, A. & FUXE, K. (1964). Evidence of the existence of monoamine-containing hormones in the central nervous system. *Acta physiol. scand.* **62**, suppl. 232.

FELDBERG, W. (1968). The monoamines of the hypothalamus as mediators of temperature responses. In *Recent Advances in Pharmacology*, ed. ROBSON, J. M. & STACEY, R. S. London: J. and A. Churchill Ltd.

GANDER, G. W., CHAFFEE, J. & GOODALE, F. (1967). Studies upon the antipyretic action of salicylates. *Proc. Soc. exp. Biol. Med.* **126**, 205–209.

HELLON, R. F. (1967). Thermal stimulation of hypothalamic neurones in unanaesthetized rabbits. *J. Physiol.* **193**, 381–395.

JACKSON, D. L. (1967). A hypothalamic region responsive to localized injections of pyrogens. *J. Neurophysiol.* **30**, 586–602.

MONNIER, M. & GANGLOFF, H. (1961). *Atlas for Stereotoxic Brain Research.* London: Elsevier.

NAKAYAMA, T. & HARDY, J. D. (1969). Unit responses in the rabbit brain stem to changes in brain and cutaneous temperature. *J. appl. Physiol.* **27**, 848–857.

ROSENDORFF, C. (1969). Studies on hypothalamic blood flow and control of thermoregulation in the rabbit. Ph.D. Thesis, University of London.

ROSENDORFF, C. & CRANSTON, W. I. (1968). Effects of salicylate on human temperature regulation. *Clin. Sci.* **35**, 81–91.

ROSENDORFF, C., MOONEY, J. J. & LONG, C. N. H. (1970). Sites of action of leucocyte pyrogen in the genesis of fever in the conscious rabbit. *Fedn Proc.* **29**, 523.