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Regional brain salicylate concentrations in afebrile and febrile rabbitts

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THERE IS increasing evidence to indicate that inflammatory fevers are mediated by a circulating endogenous pyrogen (EP) which acts directly upon cells in the anterior hypothalamus[1] and midbrain.[2] This EP, which is a polypeptide of around 14,000 mol. wt,[3] can be released by leucocytes and other cells by a variety of stimuli including bacterial pyrogens.[4] It has been suggested that the action of EP within the brain is mediated by prostaglandins of the E series, in particular prostaglandin E_1[5].

Salicylates reduce the temperature of febrile (but not afebrile) animals by acting upon the same regions of the brain as EP.[6] Vane[7] has shown that salicylates will inhibit prostaglandin synthesis in guinea-pig lung homogenates and has suggested that the antipyretic action of the drug is produced by the same mechanism occurring within the central nervous system. Such a hypothesis depends, in part, on demonstrating that brain salicylate concentrations during antipyresis are sufficient to inhibit prostaglandin synthesis. The present study was performed in order to investigate this.

METHODS

Five afebrile and five febrile New Zealand white rabbits weighing 2–3 kg were used in these experiments. They were restrained in conventional headstocks and temperature was measured with rectal thermistors advanced 8–10 cm. Fever was induced by an intravenous priming injection (2 ml) followed by a sustaining infusion for 4 hr of homologous plasma containing EP,[8] which caused a rise in temperature of 1·0–1·5° over the first hour. Thereafter temperature remained stable for as long as the EP infusion continued. Both febrile and afebrile animals each received 50 mg/kg ^{14}C-salicylate (carboxylic label, Radiochemical Centre, Amersham, England) at an approximate specific activity

of 0·5 μCi/mg in 10 ml 0·9% (w/v) sodium chloride by intravenous injection. Two aliquots of the injected solution were stored at −20° for subsequent estimation of specific activity. Thirty minutes after the administration of salicylate, the animals were anaesthetized with pentobarbitone (30 mg/kg). Twenty ml blood was withdrawn by cardiac puncture and the brains removed as rapidly as possible. The anterior hypothalamus, posterior hypothalamus, midbrain and approx. 100 mg neocortex were dissected out separately, weighed and heated in an oven at 100° to constant weight.

Salicylate was extracted from brain in 80% (w/v) ethanol as described by Sturman *et al.*[9] After removal of the solvent under reduced pressure in a rotary evaporator at 30° the residue was redissolved in 5 ml 80% (w/v) ethanol. Total ^{14}C-radioactivity was determined by liquid scintillation spectrometry (Packard Instrument Co.) using an automatic external standard and a previously constructed quench-curve. Recovery of ^{14}C-salicylate from homogenized brain was 94·2 ± 1·2 per cent. In a pilot experiment, two animals (one febrile) each received 50 mg/kg ^{14}C-salicylate (sp. act. 10 μCi/mg) intravenously and the brains removed after 30 min. ^{14}C-salicylate was extracted from the four brain regions as outlined above and the solvent removed under reduced pressure in a rotary evaporator. The residue was redissolved in 5 ml 80% (w/v) ethanol and 100 μl applied as a narrow band to 5 × 20 cm silica gel thin-layer chromatography plates (Merck) which were subsequently developed with benzene, ether, acetic acid, methanol (120:60:18:1).[10] The plates were divided into 1·5 cm bands and each band scraped into separate liquid scintillation vials containing 10 ml Instagel for subsequent determination of total ^{14}C-radioactivity. In these pilot studies, all the ^{14}C-radioactivity extracted from brain chromatographed in an identical manner to authentic ^{14}C-salicylate indicating that the central nervous system did not contain detectable amounts of salicylate metabolites. Similar findings have been reported for mice after intraperitoneal injections of ^{14}C-salicylic acid.[9] Regional brain salicylate concentrations were therefore determined from the total ^{14}C-radioactivity in the brain. Plasma salicylate concentrations were determined after adding 2 ml 0·4 M perchloric acid to 1 ml plasma. Following centrifugation, 50 μl supernatant was applied to thin-layer chromatography plates (see above) as a narrow band and estimation of ^{14}C-salicylate radioactivity performed.

In a further group of experiments the blood content of the anterior hypothalamus, posterior hypothalamus, midbrain and cortex of six febrile and six afebrile animals was measured using a technique based on that of Sterling and Gray.[11] Fever was induced as described previously and 10 ml blood was withdrawn from a central ear artery into a heparinized syringe. The plasma was separated by centrifugation and discarded. The cells were resuspended in 0·9% (w/v) sodium chloride containing 300 μCi ^{51}Cr-chromate (sodium salt, Radiochemical Centre, Amersham, England). This suspension was incubated at 37° for 15 min, the cells washed three times in 0·9% (w/v) sodium chloride, resuspended in this solution and injected intravenously into the animals. Ten min later they were anaesthetized with 30 mg/kg pentobarbitone. Five ml blood was withdrawn by cardiac puncture and the brains removed. The four brain regions under study were dissected out and weighed. The various brain regions and whole blood (200 μl) were dissolved in 5 ml concentrated sulphuric acid before the determination of total ^{51}Cr-radioactivity using an automatic gamma counter. After appropriate corrections for radioactive decay, the blood content of the various brain regions was calculated.

RESULTS AND DISCUSSION

The regional distribution of blood in the central nervous system of afebrile and febrile animals was studied in order to determine accurately the tissue concentration of salicylate. The values obtained in afebrile animals (see Table 1) are comparable to those observed by others[12] in rabbit whole brain,

TABLE 1. REGIONAL BRAIN BLOOD CONTENT OF AFEBRILE AND FEBRILE RABBITS

	Blood content (mg/g)*		
	Afebrile	Febrile	Student's *t*-test afebrile vs febrile
Anterior hypothalamus	10·4 ± 0·9	20·0 ± 4·4	2·16†
Posterior hypothalamus	9·3 ± 0·9	18·6 ± 4·2	2·18†
Midbrain	11·1 ± 1·6	15·6 ± 3·1	1·29‡
Cortex	19·2 ± 1·2	27·2 ± 6·4	1·23‡

* Calculated as mean ± S.E. of mean.
† $P < 0·05$. ‡ $P > 0·10$.

but the blood content of the cortex was significantly greater than the other areas studied ($F = 18{\cdot}03$, $P < 0{\cdot}005$). In febrile animals there was a tendency for an increase in blood content of all regions studied although this only reached conventional levels of statistical significance in the anterior and posterior hypothalamus ($t = 2{\cdot}16$, $P < 0{\cdot}05$; $t = 2{\cdot}18$, $P < 0{\cdot}05$ respectively). The increased blood content of these areas of the brain may be related to the increased hypothalamic blood flow which has been observed during fever.[13]

The regional brain tissue water salicylate concentrations in afebrile and febrile animals are shown in Table 2. These concentrations have been corrected for recovery and for blood content using the

TABLE 2. REGIONAL BRAIN TISSUE WATER SALICYLATE CONCENTRATION OF AFEBRILE AND FEBRILE RABBITS (Mean ± S.E. of Mean)

	Salicylate concn* (μg/ml)		Student's *t*-test afebrile vs febrile
	Afebrile	Febrile	
Anterior hypothalamus	11·4 ± 1·9	12·8 ± 1·4	0·61†
(range)	(7·1–17·2)	(8·0–15·9)	
Posterior hypothalamus	11·3 ± 2·7	13·0 ± 1·1	0·59†
(range)	(5·5–18·4)	(10·3–15·3)	
Midbrain	9·9 ± 2·6	10·2 ± 1·5	0·09†
(range)	(6·4–16·3)	(6·7–13·2)	
Cortex	12·0 ± 2·4	13·5 ± 1·3	0·55†
(range)	(6·3–18·3)	(11·0–17·7)	

* Calculated as mean ± S.E. of mean.
† $P > 0{\cdot}10$.

data in Table 1 and they are comparable with values obtained in whole brains from mice[9] and rats.[14] There were no significant differences amongst either afebrile or febrile brains in the regional distribution of salicylate and although there was a tendency for higher regional salicylate concentrations in brains from febrile animals, this was not statistically significant. Plasma salicylate concentrations in afebrile (mean 227 μg/ml, S.E. of mean ± 4·1 μg/ml, range 216–242 μg/ml) and febrile (mean 208 μg/ml, S.E. of mean ± 12·8 μg/ml, range 184–255 μg/ml) animals were not statistically significant.

The results of these experiments are of interest for three reasons. In the first place the mean salicylate concentrations in the anterior and posterior hypothalamus were 12·8 and 10·2 μg/ml brain water respectively, 30 min after an intravenous injection of 50 mg/kg in febrile rabbits. Similar salicylate doses producing comparable plasma salicylate concentrations have been shown to produce antipyresis in febrile animals under identical experimental conditions[8] by acting within these two areas of the brain.[6] Acetylsalicylic acid, at concentrations of 11·0 μg/ml has recently been demonstrated to produce 50 per cent inhibition of rabbit brain prostaglandin synthesis *in vitro*.[15] Although salicylate itself is ineffective as an inhibitor of prostaglandin synthetase *in vitro*, it is equipotent with acetylsalicylic acid *in vivo*.[16] The brain concentrations observed in the present study may, therefore, be sufficient to inhibit prostaglandin synthesis during antipyresis. Secondly, the absence of a significant increase in hypothalamic salicylate concentrations, despite an increase in blood flow during fever,[13] is at variance with the conclusions of Goldberg *et al.*[17] These workers showed that increasing cerebral blood flow by carbon dioxide inhalation produced a rise in the rate of salicylate penetration into the brain of rabbits. However, it is possible that in their experiments this was due to the fall in arterial pH and consequent rise in the non-ionized fraction of salicylate that accompanies carbon dioxide administration.[18] Finally, the results obtained in the present experiments indicate that a two- to three-fold range of brain salicylate concentrations exists for all regions studied despite only a small range of plasma salicylate concentrations. Within individual animals, however, the variation between regions studied was small. Thus analysis of variance showed that there was a significant difference in salicylate concentrations between animals ($F = 45{\cdot}15$, $P < 0{\cdot}005$) but not between regions ($F = 0{\cdot}71$, $P > 0{\cdot}10$). The source of this variability is unclear. Although only unbound drug in the plasma is available for penetration into the central nervous system[19] it is unlikely that differences in plasma protein binding could account for these changes. It has been shown that choroid plexus from rabbits will concentrate salicylate *in vitro*.[20] Since some weak organic acids, such as salicylate, may leave

the central nervous system by drainage into the cerebrospinal fluid and transport across the choroid plexus,[19] interindividual differences in the activity of the latter mechanism could account for these observations. Alternatively, differences in binding to macromolecules within the brain might be responsible for the variability in brain salicylate concentrations.

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Effects of some isoquinoline compounds and certain derivatives on brain phosphodiesterase activity

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RECENT investigations have shown that endogenous formation of certain isoquinoline compounds may occur in the central nervous system,[1,2] where they could have a physiological role. Several isoquinoline derivatives are also known to affect various excitable tissues, including the nervous system.[3–6]