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THE EFFECT OF VARIOUS AMINE-DEPLETING DRUGS ON THE FEVER RESPONSE EXHIBITED BY RABBITS TO BACTERIAL OR LEUCOCYTE PYROGEN

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1 The concentration of noradrenaline, dopamine and 5-hydroxytryptamine (5-HT) in the rabbit brainstem was measured during fevers produced by either an injection of bacterial pyrogen (BP) or continuous infusion of leucocyte pyrogen (LP).

2 Both procedures had little effect on the concentration of noradrenaline in the preoptic/hypothalamic area but significantly ($P < 0.001$) lowered the concentration of noradrenaline in the midbrain and pons/medulla.

3 BP significantly ($P < 0.01$) lowered the concentration of 5-HT in the preoptic/hypothalamic area but had no effect in the midbrain or pons/medulla, whereas LP significantly ($P < 0.01$) lowered the concentration of 5-HT in the midbrain and pons/medulla but had little effect in the hypothalamus.

4 The concentration of dopamine throughout the brainstem was little affected by either BP or LP fevers. However the concentration in the midbrain was significantly reduced by LP ($P < 0.001$).

5 α-Methyltyrosine (200 mg/kg) pretreatment diminished the pyrogenic response to both BP and LP whilst *p*-chlorophenylalanine (300 mg/kg) slightly enhanced the response to both forms of challenge.

6 Reserpine (1 mg/kg) diminished both types of fever whilst a combination of α-methyltyrosine and *p*-chlorophenylalanine slightly enhanced the fevers produced by either BP or LP.

7 The results obtained are discussed in relation to the mechanisms involved in the production of fever and to the possible function of noradrenaline and 5-HT as thermoregulatory transmitters.

Introduction

It has long been recognized that the intravenous injection of certain substances produce fever both in man and in animals and the term pyrogen has been traditionally used to describe these substances. Although it is now commonly accepted that bacterial (or exogenous) pyrogen fever is ultimately caused by leucocyte (or endogenous) pyrogen (Atkins & Wood, 1955a, b), there is still controversy concerning a site of action for pyrogen. Cooper, Cranston & Honour (1967) injected pyrogens directly into rabbit brain in an attempt to identify the site of action. They concluded that bacterial pyrogen provoked the release of leucocyte pyrogen which in turn acted on the anterior hypothalamus. More recently, Rosendorff & Mooney (1971) produced fever by injecting leucocyte pyrogen into the lower brain stem from which they concluded that there may be more than one site of action.

The theory that pyrogens produce fever indirectly by the release of hypothalamic noradrenaline or 5-HT was first suggested by Feldberg & Myers (1963). Subsequent experiments, designed to test the possible involvement of these amines in the genesis of fever, have provided equivocal results. Thus reserpine has been shown to protect rabbits from bacterial pyrogen fever (Göing, 1959; Kroneberg & Kurbjeweit, 1959; Des Prez, Helman & Oates, 1966) although Cooper *et al.* (1967) found that intraventricular administra-

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tion of reserpine did not suppress bacterial pyrogen fever. Similarly, Des Prez *et al.* (1966) found that bacterial pyrogen fever was not associated with changes in hypothalamic noradrenaline or 5-HT concentrations whereas Canal & Ornesi (1961); Kuruma, Takagi & Yanada (1964) and Takagi & Kuruma (1966) all found hypothalamic 5-HT stores to be depleted. Giarman, Tanaka, Mooney & Atkins (1968) found that both amines were depleted. Feldberg (1968) has sought to resolve these discrepancies and also the observation that 5-HT injected directly into the CNS lowers rather than raises body temperature in rabbits (Cooper, Cranston & Honour, 1965) by suggesting that the amines may act as thermoregulatory transmitters at additional sites caudal to the hypothalamus.

The present experiments were undertaken (a) to investigate the possibility that noradrenaline and 5-HT function as thermoregulatory transmitters at sites caudal to the hypothalamus (b) to compare the effects of leucocyte pyrogen and bacterial pyrogen on the cerebral concentration of these amines (c) to use specific amine depleting drugs to investigate the fever suppressing properties of reserpine.

Methods

Healthy female New Zealand White rabbits weighing 3.0-3.6 kg were used. Before use for fever experiments, these rabbits were trained for a total of 18 h (three 6 h periods) to remain in open ended wooden stocks during which time their body temperature was recorded. Temperatures (±0.05° C) were measured using a Model 44 telethermometer (Yellow Springs Instrument Company) connected to a YSI rectal probe type 401 which was inserted 100 mm into the rectum (Lomax, 1966). At the end of this training period, rabbits whose rectal temperature fell outside of the range 38.8-39.8° C were rejected. All training and fever experiments were carried out in a quiet, well-lit room where the ambient temperature ranged between 20 and 24° C.

For experiments with bacterial pyrogen, the intravenous challenge was provided by 0.1 ml/kg of TAB vaccine (Burroughs, Wellcome & Co), containing 1000 million *S. typhi*, 500 million *S. paratyphi* A and 500 million *S. paratyphi* B in each millilitre. This dose was adhered to throughout this study although it was found that there was little correlation between response and body weight. Indeed, similar results could be obtained by administering 0.3 ml TAB vaccine per rabbit, an observation noted also by Murphy (1967). Stable leucocyte pyrogen fever was achieved according to the method of Cranston, Luff, Rawlins & Rosendorff (1970) using an initial intravenous injection of 10 ml leucocyte pyrogen followed by an infusion at 0.08 ml/minute. Leucocyte pyrogen was obtained from sterile peritoneal exudates prepared according to the method of Bennet & Beeson (1953).

On an experimental day, body temperature was recorded for 60-90 min prior to pyrogen challenge and the mean of the pre-injection temperatures was taken as the base line for the subsequent calculation of fever indices. In the present study the fever responses produced by various treatments were compared by measuring the area underneath a 6 h fever curve. This area was defined as the fever index for that treatment.

Samples of brain tissue for amine assay were dissected from animals which had been killed by an intravenous injection of air. Amines were extracted and assayed as described by Metcalf (1974). Reserpine (Halewood Chemicals Ltd), α-methyltyrosine methyl ester HCl (R. Emanuel Ltd) and *p*-chlorophenylalanine methyl ester HCl (kindly donated by Pfizer, Ltd) were prepared immediately prior to use; doses of the amino acids were expressed as the parent acid in each case.

Results

The effect of pyrogen fever on the concentrations of amines in brain

(a) Bacterial pyrogen Fever was induced in rabbits by the administration of bacterial pyrogen (0.1 ml TAB vaccine/kg i.v.). The biphasic course of the subsequent fever (Figure 1a) was monitored and 3 h after dosage (i.e. at maximum fever) the animals were killed by air embolism and the amine content of the dissected hypothalamus, midbrain and pons/medulla determined (Table 1). The noradrenaline concentration in the hypothalamus was not significantly different from control animals but the concentration of this amine in both the midbrain and pons/medulla was significantly reduced ($P < 0.001$). In contrast, the 5-HT concentration in these latter areas was unaffected although the hypothalamic concentration of 5-HT was significantly reduced ($P < 0.05$). The concentration of dopamine in both the hypothalamus and the midbrain appeared unaffected by bacterial pyrogen fever.

(b) Leucocyte pyrogen A stable, long-lasting fever was induced in rabbits by a 10 ml intravenous injection of leucocyte pyrogen followed by a continuous intravenous infusion at 0.08 ml/minute. Body temperature rose steadily over 1-2 h

and then remained steady at this elevated level (Figure 1b). After 3 h when body temperate had stabilized at the raised level, the animals were killed by air embolism and the requisite brain regions dissected out and analysed for amines (Table 1). Although the concentrations of noradrenaline and 5-HT in the hypothalamus were reduced when compared with controls, in neither case was the reduction statistically significant. In contrast, the concentration of both noradrenaline and 5-HT were significantly reduced in both the midbrain and pons/medulla. Midbrain dopamine was significantly ($P < 0.001$) reduced but the concentration in both the hypothalamus and the pons/medulla was unaffected.

The effect of reserpine on the concentrations of amines and on the magnitude of pyrogen fever

The degree of amine depletion achieved by reserpine (1 mg/kg i.p.) administered to rabbits 17 h before death is shown in Table 1. With the exception of dopamine in the pons/medulla region, all three amines were depleted to a significant extent in all three regions. Rabbits pretreated with reserpine 17 h before challenge with bacterial pyrogen did not exhibit a normal febrile response (Figure 2). Similarly animals pretreated with reserpine 17 h prior to challenge with leucocyte pyrogen also exhibited a significantly diminished pyrogenic response (Figure 3). However animals pretreated with reserpine 42 h prior to leucocyte pyrogen challenge exhibited a normal febrile response to an infusion of leucocyte pyrogen (Figure 3).

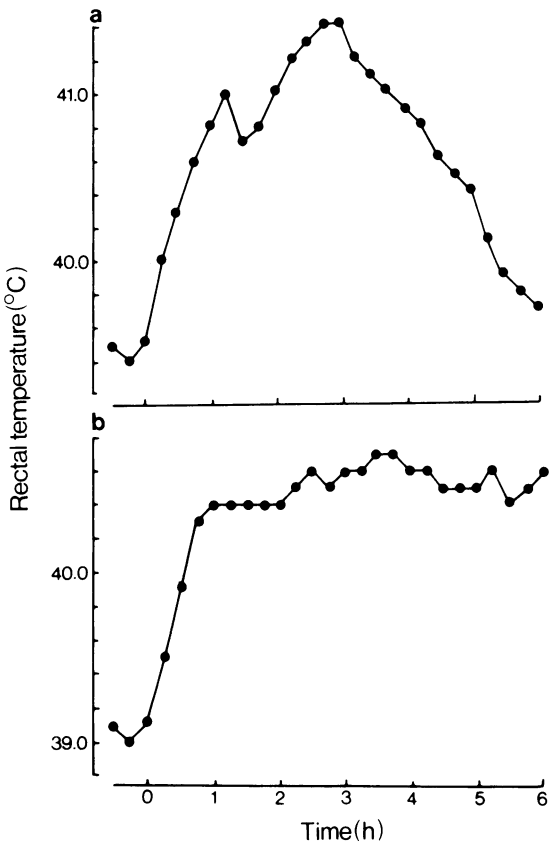
The effects of α-methyltyrosine

α-Methyltyrosine (200 mg/kg i.p.) administered as the soluble methyl ester hydrochloride was administered to rabbits 17 h before killing for amine assay. Table 1 shows that whilst 5-HT stores were apparently unaffected by the drug, both noradrenaline and dopamine were significantly depleted in all three areas of brain. Whilst α-methyltyrosine diminished the febrile response to both bacterial (Figure 2) and leucocyte pyrogen (Figure 3) the

Table 1 The effect of various treatments upon the amine concentration in several regions of rabbit brain

Treatment	*Amine*	*Hypothalamus*	*Midbrain*	*Pons/Medulla*
1. None (controls)	Noradrenaline	1.61 ± 0.06	0.73 ± 0.04	0.49 ± 0.03
	Dopamine	0.32 ± 0.03	0.42 ± 0.02	0.15 ± 0.02
	5-HT	1.38 ± 0.18	1.17 ± 0.06	0.50 ± 0.04
2. Bacterial pyrogen (0.1 ml TAB/kg i.v.)	Noradrenaline	1.48 ± 0.15	0.31 ± 0.02†	0.15 ± 0.02†
	Dopamine	0.26 ± 0.03	0.39 ± 0.10	
	5-HT	0.76 ± 0.05**	1.06 ± 0.10	0.47 ± 0.05
3. Leucocyte pyrogen fever (continuous infusion)	Noradrenaline	1.28 ± 0.14	0.27 ± 0.03†	0.27 ± 0.03†
	Dopamine	0.29 ± 0.03	0.23 ± 0.03†	0.13 ± 0.08
	5-HT	1.03 ± 0.11	0.84 ± 0.05**	0.35 ± 0.05**
4. Reserpine (1 mg/kg i.p.)	Noradrenaline	0.32 ± 0.07†	0.07 ± 0.02†	0.09 ± 0.02†
	Dopamine	0.14 ± 0.03**	0.07 ± 0.03†	0.08 ± 0.04
	5-HT	0.17 ± 0.04†	0.21 ± 0.04†	0.09 ± 0.02†
5. α-Methyltyrosine (200 mg/kg i.p.)	Noradrenaline	0.27 ± 0.03†	0.07 ± 0.02†	0.06 ± 0.01†
	Dopamine	0.18 ± 0.03*	0.24 ± 0.03**	0.08 ± 0.01*
	5-HT	1.34 ± 0.05	1.32 ± 0.06	0.59 ± 0.03
6. *p*-Chlorophenylalanine (300 mg/kg i.p.)	Noradrenaline	1.73 ± 0.18	0.56 ± 0.03*	0.37 ± 0.02*
	Dopamine	0.38 ± 0.03	0.48 ± 0.07	0.17 ± 0.03
	5-HT	0.22 ± 0.02†	0.40 ± 0.04†	0.17 ± 0.02†
7. α-Methyltyrosine (200 mg/kg i.p.) + *p*-Chlorophenylalanine (300 mg/kg i.p.)	Noradrenaline	0.24 ± 0.04†	0.08 ± 0.03†	0.08 ± 0.02†
	Dopamine	0.15 ± 0.02**	0.10 ± 0.01†	0.04 ± 0.00**
	5-HT	0.32 ± 0.04**	0.44 ± 0.04†	0.14 ± 0.02

Values are mean values ± s.e. mean expressed as μg/g derived from 6-12 experiments.
* denotes that the value is significantly different from control values at $P < 0.05$.
** denotes significance at $P < 0.01$ and
† denotes significance at $P < 0.001$.



results were only significantly different ($P < 0.05$) from fevers in control animals in the case of leucocyte pyrogen.

The effects of p-*chlorophenylalanine*

Table 1 illustrates that whilst *p*-chlorophenylalanine (300 mg/kg i.p.) administered as the methyl ester hydrochloride 72 h prior to death, significantly lowered the concentration of 5-HT in all three brain regions ($P < 0.001$), it also lowered the concentration of noradrenaline in the brainstem ($P < 0.05$). *p*-Chlorophenylalanine when administered prior to pyrogen challenge did not alter the febrile response to bacterial (Figure 2) or leucocyte pyrogen (Figure 3) to a significant extent, although in the latter case the response was slightly enhanced when compared with control animals.

The combined effect of α-methyltyrosine and p-*chlorophenylalanine*

Seventy-two hours after *p*-chlorophenylalanine (300 mg/kg i.p.) and 17 h after α-methyltryrosine

Figure 1 Typical fevers produced by the administration of bacterial or leucocyte pyrogen. (a) At zero time, 0.1 ml/kg TAB vaccine injected intravenously, (b) at zero time, 10 ml leucocyte pyrogen injected intravenously followed by a continuous infusion of 0.08 ml/minute.

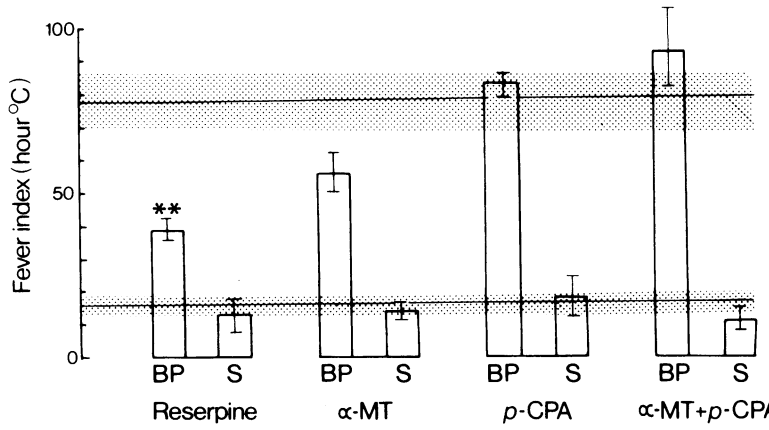


Figure 2 The effect of various drug treatments on the fever induced by injection of bacterial pyrogen (BP) in rabbits. Fever indices are expressed as mean (hours ° C) ± s.e. mean obtained from 6-8 animals. The shaded areas represent (mean ± s.e. mean) the fever response of untreated animals to BP challenge (upper) and saline injection (lower). Histograms represent the response to pyrogen challenge (BP) or saline injection (S) after drug pretreatment. Drug dosage and time prior to challenge: reserpine 1 mg/kg i.p., 17 h; α-methyltyrosine (α-MT) 200 mg/kg i.p., 17 h; *p*-chlorophenylalanine (*p*-CPA) 300 mg/kg i.p., 72 h; α-MT + *p*-CPA—combined α-methyltyrosine and *p*-chlorophenylalanine pretreatments. ** Indicates results significantly different from controls at $P < 0.01$.

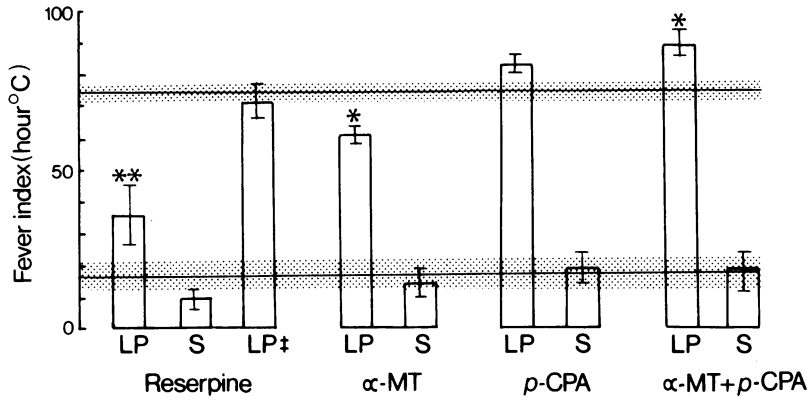


Figure 3 The effect of various drug treatments on the fever induced by a continuous infusion of leucocyte pyrogen (LP). Fever indices are expressed as mean (hours °C) ± s.e. mean obtained from 6-13 animals. The shaded areas represent (mean ± s.e. mean) the fever response of untreated animals to LP challenge (upper) and saline infusion (lower). Histograms represent the response to pyrogen challenge (LP) or saline infusion (S) after drug pretreatment. Drug dosage and time prior to challenge: reserpine 1 mg/kg i.p., 17 h; group marked ‡ received reserpine 42 h before challenge; α-methyltyrosine (α-MT) 200 mg/kg i.p., 17 h; *p*-chlorophenylalanine (*p*,-CPA) 300 mg/kg i.p., 72 h; α-MT + *p*-CPA—combined α-methyltyrosine and *p*-chlorophenylalanine pretreatments. * and ** indicate results significantly different from controls at $P < 0.05$ and $P < 0.001$ respectively.

Table 2 The effect of amine depleting drugs on rectal temperature in the rabbit prior to pyrogen challenge

Drug	*Dose (mg/kg i.v.)*	*Hours since dosing*	*Rectal temp. °C*
Control	–	–	39.4 ± 0.1
Reserpine	1	17	37.3 ± 0.5*
Reserpine	1	42	38.6 ± 0.1**
α-Methyltyrosine	200	17	38.7 ± 0.1**
p-Chlorophenylalanine	300	72	39.7 ± 0.2
α-Methyltyrosine+	200	17	39.2 ± 0.2
p-Chlorophenylalanine	300	72	

Temperatures are mean ± s.e. mean obtained from 6-13 animals * and ** indicate results are significantly different from controls at $P < 0.01$ and $P < 0.001$ respectively.

(200 mg/kg i.p.), both administered as the methyl ester hydrochlorides, a group of rabbits was killed and portions of the brain analysed for amines (Table 1). All three amines were depleted in the three areas analysed. The extent of amine depletion achieved by the combined treatment was not significantly different from that achieved by reserpine. When animals pretreated with both α-methyltyrosine and *p*-chlorophenylalanine were subsequently subjected to pyrogen challenge the ensuing febrile response was slightly enhanced in both the case of bacterial pyrogen (Figure 2) and leucocyte pyrogen (Figure 3) although only in the latter case was the difference significant ($P < 0.05$).

The effect of amine depleting drugs on body temperature

The effect produced by the various amine depleting treatments prior to pyrogen administration was recorded. The results (Table 2) show that both reserpine and α-methyltyrosine produced significant hypothermia when compared with control animals.

Discussion

Feldberg & Myers (1963) suggested that pyrogen fever is mediated by the release of biogenic amines

from the anterior hypothalamus. Since that time observations made by other workers have raised the possibility that the ratio of cations in the hypothalamus (Feldberg, Myers & Veale, 1970) or the release of prostaglandin E_1 (Milton & Wendlandt, 1970) from the same structure may also be important for the chemical mediation of fever.

Several biochemical investigations undertaken since 1963 (Canal & Ornesi, 1961; Kurumma *et al.*, 1964; Takagi & Kuruma, 1966) have supported the original suggestion of Feldberg & Myers by demonstrating that the concentration of 5-HT in the hypothalamus is lowered during bacterial pyrogen fever. The present results confirm that hypothalamic 5-HT is significantly depleted during bacterial pyrogen fever but not during leucocyte pyrogen fever. In contrast, 5-HT in both the midbrain and pons/medulla is reduced during leucocyte but not during bacterial pyrogen fevers. There is therefore no common response of the 5-HT levels to the two pyrogens and since depletion of brain 5-HT by *p*-chlorophenylalanine did not affect the fever produced by the two pyrogens no strong evidence has been obtained for a role of 5-HT in fever heat production.

The effects of the two pyrogens on brain noradrenaline were more comparable. Both pyrogens significantly reduced the concentration of noradrenaline in the brain stem but left that in the hypothalamus unaffected. Also α-methyltyrosine, which depleted noradrenaline in all areas, reduced the fever produced by both pyrogens and itself caused a significant hypothermia. Thus hypothalamic noradrenaline may well be important for the heat production which is achieved in pyrogen fever. A rise in body temperature could then follow the loss of some balancing factor affected by the pyrogens such as midbrain noradrenaline (both pyrogens), hypothalamic 5-HT (bacterial pyrogen) or brain stem 5-HT (leucocyte pyrogen). Such a role for 5-HT is supported by the slight hyperthermia caused by 5-HT depletion after *p*-chlorophenylalanine and it may well be that it is the ratio of 5-HT to noradrenaline in the hypothalamus and brain stem that is important in the control of body temperature as originally suggested by Feldberg & Myers (1963).

Several points arise from the experiments in which the ability of amine depleting drugs to interfere with pyrogen fever was tested. Reserpine suppressed the febrile response to both bacterial and leucocyte pyrogens given 17 h after the drug, but the ability of animals to respond normally to leucocyte pyrogen was restored within 42 hours. However, the role of brain amines in reserpine suppression of fever must be uncertain since after α-methyltyrosine and *p*-chlorophenylalanine together, the febrile responses to both types of pyrogen were enhanced rather than suppressed although the drug combination depleted brainstem amines to the same extent as reserpine. Thus the fever suppression produced by reserpine may depend on pharmacological properties of the molecule other than its ability to deplete tissue stores of catecholamines or 5-HT and the results obtained in these experiments illustrate the dangers of using reserpine as a specific biochemical tool to investigate physiological responses supposedly controlled by biogenic amines. Fever represents a state of abnormal thermoregulation and the present results obtained with amine depleting drugs suggest that the genesis of pyrogen fever is a complex pathophysiological reaction which probably involves other chemical mediators in addition to brainstem monoamines.

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