Pyrogen Fever in Rabbits Pretreated with *p*-Chlorophenylalanine or 5,6-Dihydroxytryptamine

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Summary. The hyperthermic effect of a bacterial pyrogen has been studied in rabbits pretreated or not with *p*-chlorophenylalanine or 5,6-dihydroxytryptamine. The results obtained indicate that a selective reduction of cerebral 5-hydroxytryptamine levels by these drugs do not significantly affect pyrogen hypertermia.

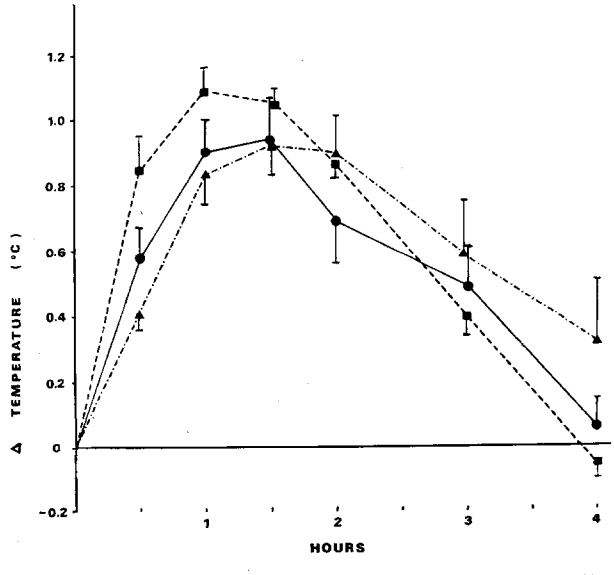
Several pieces of evidence favour the hypothesis that 5-hydroxytryptamine (5-HT) may be involved in the central regulation of body temperature[2–10]. Conflicting reports have appeared, however, on the role of 5-HT in the febrile response induced by pyrogens.

Giarman et al.[11] and Mašek, Rašková and Rotta[12] have suggested that, in rabbits, the release of 5-HT may limit the fever induced by pyrogen, while other authors claimed that serotonin mediates pyrogen fever[3, 13, 14]. Furthermore, Jacob, Girault and Peindaries[15] and Peindaries and Jacob[16] reported that 5-HT may have opposite effects on the thermic response in rabbits and pointed out how this dualistic feature of the central action of 5-HT may solve the apparent discrepancy.

In the work here reported, we have investigated whether the functional impairment of central 5-HT activity induced by either blockade of 5-HT synthesis through *p*-chlorophenylalanine (PCPA)[17], or selective destruction of serotoninergic nerve terminals by 5,6-dihydroxytryptamine (5,6-DHT) treatment[18–21], might affect the hyperthermic action of pyrogens.

Materials and methods. Male Hasen Füllinsdorf rabbits (2–3 kg of body weight) were used with a basal temperature between 38 and 40 °C, which was stable throughout the day.

Rabbits were divided into 3 groups of 12 animals each. 1 group was injected only with saline. The 2nd group, before receiving pyrogen injection, was given i. p. *p*-chlorophenylalanine methyl ester (Regis) (100 mg/kg daily for 4 days). Another group was injected with 5,6-dihydroxytryptamine creatinin sulfate[22] (50 µg base/50 µl 0.9% NaCl containing 0.1% ascorbic acid) into both cerebral ventricles 3 days before the experiment.



Changes in rectal temperature induced by Pyrifer VII (0.0005 ml/kg i.v.) in control (●—●), PCPA (100 mg/kg i.p. daily for 4 days) (■ - - ■) or 5,6-DHT (50 µg into each ventricle) (▲·····▲) pretreated rabbits. Each point represents the mean ± SE of 12 determinations.

Intraventricular injections were made with a direct puncture technique. After having drilled a small hole in the skull 2 mm laterally to the midline and 1 mm rostrally to the bregma, a 6.5 mm long needle mounted on a Hamilton microsyringue was inserted vertically and a volume of 50 µl was injected. In order to prevent leakage of injection fluid, before extraction of the needle, a drop of paste (Histoacryl-N-blau B. Braun, Melsungen) was placed all around the needle.

On the day of the experiment at 09.00 h all the rabbits were injected via the marginal ear vein with 0.0005 ml/kg of Pyrifer VII[23] (containing pyrogenic substances from nonpathogenic bacteria). Temperature was recorded continuously by a termistor probe inserted 5 cm into the rectum and connected with a temperature recorder (from Texas Instruments Inc.).

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Effect of PCPA (100 mg/kg i.p. daily for 4 days) or 5,6-DHT (50 µg into each ventricle) on monoamine concentrations in the hypothalamus and residual brain of the rabbit

Treatment	5-HT (µg/g ± SE)		NE (µg/g ± SE)		DM (µg/g ± SE)	
	Hypothalamus	Rest of the brain	Hypothalamus	Rest of the brain	Hypothalamus	Rest of the brain
Controls	0.97 ± 0.048	0.46 ± 0.026	1.20 ± 0.050	0.30 ± 0.20	0.23 ± 0.010	0.21 ± 0.010
PCPA	0.29 ± 0.010[b]	0.19 ± 0.006[b]	1.07 ± 0.171	0.22 ± 0.008[a]	0.23 ± 0.057	0.18 ± 0.009
5,6-DHT	0.58 ± 0.001[b]	0.30 ± 0.020[b]	1.10 ± 0.120	0.25 ± 0.009	0.28 ± 0.044	0.18 ± 0.010

Each value is the mean ± SE of 6 determinations. [a] $P \leqslant 0.05$; [b] $p \leqslant 0.001$ relative to controls.

Five h after pyrogen administration, the animals were killed by decapitation, the brains were removed and in some cases (see results) the hypothalamus was dissected out. Catecholamines were estimated fluorometrically in the whole brain or in the hypothalamus according to the method of SHELLEMBERGER and GORDON[24]. For 5-HT determinations in blood platelets, some of the animals were exsanguinated under slight ether anesthesia through a polyethylene cannula placed in the carotid artery and blood platelets were isolated as described by DA PRADA and PLETSCHER[25,26]. Significance of differences between groups was calculated by the Student's *t*-test and a probability of $p \leq 0.05$ was accepted as statistically significant.

Results and discussion. PCPA or 5,6-DHT treatment, per se, did not alter the normal temperature of the rabbits; in fact, immediately before pyrogen injection, the basal temperature of the control animals was 39.18 °C, while animals which received PCPA or 5,6-DHT had a mean value of 39.09 and 38.83 °C respectively ($p > 0.05$).

In contrast to the results obtained with PCPA by GIARMAN et al.[11] and MAŠEK, RAŠKOVÁ and ROTTA[12], the Figure shows how, under our experimental conditions, PCPA as well as 5,6-DHT pretreatment was unable to modify the hyperthermic response induced by Pyrifer VII. The levels of the amines were determined in the hypothalamus, in the rest of the brain and, in the case of 5-HT, also in blood platelets, 5 h after pyrogen administration.

The results are shown in the Table: 5,6-DHT reduced the 5-HT content in the hypothalamus and in the rest of the brain to about 60% of the control value, whereas the norepinephrine and dopamine levels were only slightly modified. The central serotonin depletion induced by PCPA treatment was also quite specific and even more pronounced (60–70% depletion). Furthermore, PCPA was able to reduce the 5-HT levels in blood platelets from $97 \times 10^{-6} \pm 10$ to $35 \times 10^{-6} \pm 6$ mmoles/mg protein (64% depletion).

In summary, our results do not seem to support the hypothesis that 5-HT plays an important role in the hyperthermic response induced by pyrogens in rabbits. In fact, a selective reduction of 5-HT hypothalamic levels induced by PCPA or by 5,6-DHT pretreatments, did not modify significantly pyrogen hyperthermia.

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Altered Brain Cyclic AMP-Responses in Rats Reared in Enriched or Impoverished Environments

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Summary. The accumulation of radioactive cyclic AMP elicited by various neurohormones has been examined in adenine-labeled telencephalon slices from rats raised in enriched or impoverished environments. Basal levels of cyclic AMP and responses of the brain slice cyclic AMP-generating systems to norepinephrine, isoproterenol and adenosine did not differ between the two group of rats, while responses to prostaglandin E_1 were significantly greater with the impoverished group and responses to histamine appeared to be greater with the enriched group.

Brain cyclic AMP-generating systems have been shown to be capable of adaptive responses to chronic alterations in synaptic input[2]. Thus, when neocortical levels of the neurotransmitter, norepinephrine, are chronically depleted by reserpine, 6-hydroxy-dopamine or lesions of the medial forebrain bundle, the postsynaptic norepinephrine-sensitive cyclic AMP systems of the neocortex become hyper-responsive[3–7]. Similarly, reduction of neocortical levels of histamine and serotonin by lesions of the medial forebrain bundle results in hyper-responsiveness of histamine and probably serotonin-sensitive cyclic AMP-systems in rat cortical slices[7]. Conversely, treatments with drugs such as amphetamine, chlorpromazine, desipramine and imipramine which increase postsynaptic availability of norepinephrine result in a sub-sensitivity of brain slice cyclic AMP-systems to norepinephrine[8–11]. Thus, investigation of the responsiveness of cyclic AMP-generating systems in brain slices would appear to provide a valuable method for probing the effects of chronic drug or environmental manipulations on the synaptic activity of different neurotransmitter pathways in the central nervous system.

Rats raised in enriched versus impoverished environments have been shown to consistently differ in a number