Lack of Relationship of Febrile Response to Endotoxin and Brain Stem 5-Hydroxytryptamine (32803)

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A previous report from this laboratory demonstrated that pretreatment of rabbits with reserpine in dosage which markedly depleted both brain stem 5-hydroxytryptamine (5HT) and norepinephrine (NE) produced substantial inhibition of the febrile response to intravenous endotoxin (1). It was further demonstrated that pretreatment with either α-methyl-paratyrosine or with guanethidine, in dosages which depleted brain stem NE to a degree equal to that produced by reserpine but did not alter brain stem 5HT, did not inhibit endotoxin fever.

These studies indicated that reserpine-induced inhibition of endotoxin fever was not related to brain stem NE and were thought to be compatible with the notion that the important fever-inhibiting effect produced by reserpine was reduction of brain stem 5HT. This formulation was concordant with several studies (2–4) relating mechanisms of fever production to 5HT metabolism.

Recently it became possible to test this hypothesis by means of a compound, DL-*p*-chlorophenylalanine (*p*-CPA) which selectively depletes brain stem 5HT to very low levels while producing only slight lowering of NE (5). Studies with this compound have demonstrated that endotoxin fevers actually have no relationship to brain stem 5HT concentrations.

Materials and Methods. Fever studies, data recording, the method of obtaining a standardized section of hypothalamus, and methods for fluorometric analysis of 5HT and NE content have been previously reported in detail (1). The DL-*p*-chlorophenylalanine was generously supplied by Charles Pfizer and Company, Groton, Connecticut. A dose of 250 mg/kg of *p*-chlorophenylalanine was found to reduce serotonin in the hypothalamus to levels as low as those seen after reserpine. Accordingly, this dose was administered by the intraperitoneal route 72 hours before endotoxin.

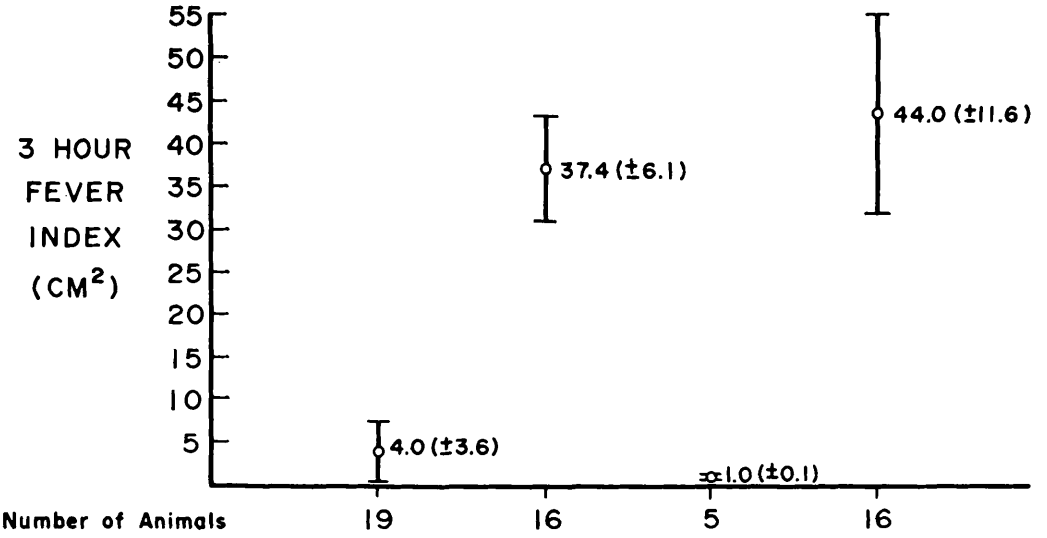
TABLE I. Effects of Various Dosages of *p*-CPA on Brain Stem 5HT.

No. of animals	*p*-CPA (mg/kg)	Brain stem 5HT	Number of animals dead before 72 hours
2	50	0.09–0.09	0
2	100	0.09–0.09	1
2	150	0.09–0.06	1
2	200	0.03–0.06	1
2	250	0.03–0.03	1
2	300	0.02–0.03	2
2	350	0.03–0	0
2	400	0 –0.03	0
2	450	0 –0	0
2	500	0 –0	0

Results and Discussion. Table I demonstrates that *p*-CPA in a dosage of 50 mg/kg i.p. produces substantial depletion of brain stem 5HT 72 hours later, and larger doses produce only slightly greater effect. A few deaths occurred in the rabbits receiving *p*-CPA without a relation to the dose given. The animals had some diarrhea but otherwise appeared normal till death or sacrifice.

Figure 1 demonstrates that *p*-CPA in the dosage administered produced depletion of 5HT to a degree equal to that produced by reserpine, but did not inhibit febrile responses to endotoxin. Reserpine significantly antagonized the febrile effect of endotoxin when 5HT was reduced to comparable levels (1). This eliminates the possibility that febrile responses to endotoxin are related to hypothalamic 5HT levels and suggests that the hypothermic properties of reserpine are due to effects other than the depletion of brain stem 5HT and NE produced by this drug.

Summary. Pretreatment of rabbits with *p*-CPA in dosage sufficient to deplete over 90% of brain stem 5HT does not modify febrile responses to bacterial endotoxin.



Number of Animals	19	16	5	16
PRETREATMENT (72 Hrs. Before Challenge)	Saline I.P.	Saline I.P.	PCPA (250 mg/Kg I.P.)	PCPA (250 mg/Kg I.P.)
CHALLENGE	Saline I.V.	Endo. 25 μg I.V.	Saline I.V.	Endo. 25 μg I.V.
BRAIN STEM 5HT (μg/gm)	0.51±.15	0.48±.11	0.06±.06	0.05±.02
BRAIN STEM NE (μg/gm)	0.53±.11	0.48±.09	0.39±.11	0.53± 1.0

FIG. 1.

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Freezing, Storage, and Thawing of Mouse Leukemic Cells, L5178Y, in Culture* (32804)

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The general conditions needed to preserve mammalian cells successfully have been reviewed by several authors (1–4). In routine use of preservation by freezing, only a small number of cells need to survive in order to

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