abortive infection of hamster cells with adeno-12 is suggested by a) decreased mitotic index but increased DNA synthesis induced by either adeno-5 or adeno-12, and b) association of the incorporated DNA-label with the viral intranuclear inclusions of hamster cells infected with adeno-5.

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Inhibtion of Endotoxin Fever by Reserpine.* (31242)

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The present studies demonstrate that treatment of rabbits with reserpine inhibits the febrile response to bacterial endotoxin injected 24 hours later, and explore the relationship of this inhibition to reserpine-induced depletion of brain stem 5-hydroxytryptamine (5HT) and norepinephrine (NE), both of which have been implicated in temperature control mechanisms(1,2).

Materials and methods. Adult male albino rabbits were trained to tolerate restraints and rectal temperature probes for 8-hour periods on at least 3 consecutive days. Temperatures were recorded every 10 minutes (Telethermometer, Yellow Springs Instrument Corp., Yellow Springs, Ohio) during the training period and for at least 3 hours before pyrogen challenge. Only animals achieving a stable baseline temperature were used. All experiments were performed in an inside, windowless room in which temperature varied little from 20°C. Temperature was charted on coordinate paper using scales of 4 cm per degree C on the ordinate and 3 cm per hour on the abscissa. The area between the baseline temperature and the fever curve over a 3-hour period following pyrogen challenge was determined by planimetric integration and expressed as the fever index (FI) in cm^2. An endotoxin of the Boivin type (Difco *E. coli* 0127 B:8) was obtained from commercial sources, suspended in pyrogen-free saline, and used in the indicated dosage. In general, fairly high dosage (25 μg) was employed to facilitate recognition of fever inhibition. Ani-

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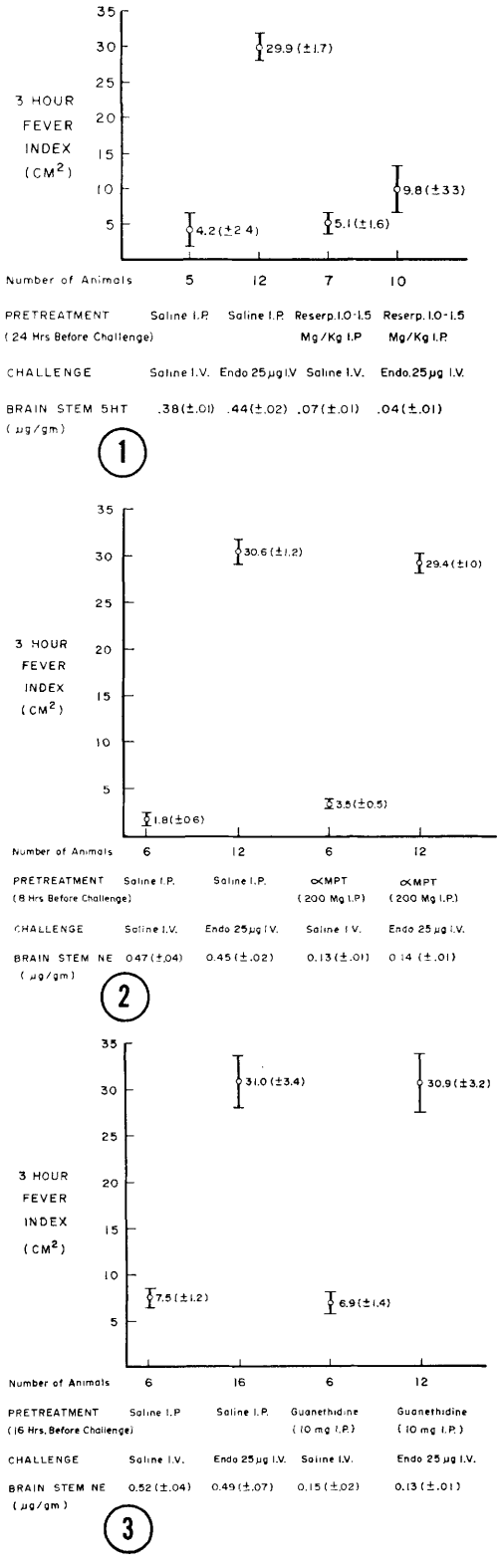

FIG. 1. Effect of reserpine on endotoxin fever and brain stem 5HT. Figures in parenthesis are standard error of mean.

FIG. 2. Effect of alpha-methyl-paratyrosine on endotoxin fever and brain stem NE. Figures in parenthesis are standard error of mean.

FIG. 3. Effect of guanethidine on endotoxin fever and brain stem NE. Figures in parenthesis are standard error of mean.

mals were sacrificed at the end of the experimental observations, the brain quickly removed, and that portion of the hypothalamus extending from the posterior border of the optic chiasm anteriorly to the anterior border of the mammillary bodies posteriorly was removed, making the lateral cuts 0.5 cm from the midline so as to converge and provide a wedge of tissue approximately one cm deep. This tissue was stored at minus 40°C until used for determination of 5HT and NE content. These determinations were carried out according to the methods of Udenfriend *et al* (3) and Shore and Olin(4) using the Farrand Photofluorometer (Farrand Optical Co., New York). Reserpine was obtained from Vitamix Pharmaceuticals, Philadelphia, Pa., and 5-hydroxy-*DL*-tryptophan from Nutritional Biochemicals, Cleveland, Ohio. The following compounds were generously supplied to the authors: alpha-methyl-paratyrosine by the Merck Sharp & Dohme Research Laboratories, West Point, Pa.; serotonin creatinine sulfate and methysergide by Sandoz Pharmaceuticals, Hanover, N. J.; and guanethidine sulfate by CIBA Pharmaceutical Co., Summit, N. J.

Results. Administration of endotoxin to rabbits produces a significant febrile response which is not associated with changes in the concentration of either 5HT or NE in the hypothalamus (Table I).

Fig. 1 presents the results of the series of experiments in which rabbits were pretreated with either saline or reserpine 24 hours before endotoxin challenge. Preliminary observations demonstrated that reserpine in dosage of 0.5 mg per kg depressed brain stem 5HT to one-third of control values but inhibited febrile responses to endotoxin only slightly. However, when reserpine dosage was increased to 1 or 1.5 mg per kg, brain stem 5HT was lowered to less than 15% of control values and

TABLE I. Effects of Various Agents on Hypothalamic 5HT & NE.*

Treatment	5HT, μg/g brain	NE, μg/g brain
Saline	.49 (.01)	.55 (.04)
Endotoxin, 25 μg I.V. 3 hr before sacrifice	.43 (.01)	.50 (.09)
Reserpine, 1 mg/kg I.M. 24 hr before sacrifice	.05 (.01)	.17 (.03)
Alpha-methyl-paratyrosine, 200 mg I.P. 8 hr before sacrifice	.46 (.03)	.13 (.01)
Guanethidine, 10 mg/kg I.P. 16 hr before sacrifice	.72 (.05)	.15 (.02)

* Numbers in parentheses represent standard error of mean.

clear-cut suppression of endotoxin fever was observed. In several instances endotoxin injection was followed by an actual fall in temperature. These hypothermic responses were not taken into account in the values recorded in Fig. 1, all negative values being considered as zero.

Reserpine in dosage of 1 mg per kg also significantly lowers hypothalamic NE concentrations (Table I). Accordingly, a similar series of observations were undertaken utilizing pretreatment with alpha-methyl-p-tyrosine (AMPT), an agent known to deplete norepinephrine selectively both in the central nervous system and in peripheral stores (5), probably by inhibiting hydroxylation of tyrosine to dihydroxyphenylalanine(6). It was found that AMPT depleted brain stem NE to approximately the same degree as observed following reserpine (Table I), but, in contrast to reserpine, did not lower brain stem 5HT. Fig. 2 illustrates that AMPT in this dosage does not modify the febrile response to injected endotoxin.

To investigate the possibility that peripheral adrenergic neuron blockade was responsible for the inhibition of endotoxin fever by reserpine, similar studies were carried out utilizing pretreatment with guanethidine. In previous studies, guanethidine has been found to block peripheral adrenergic neurons selectively without depletion of brain NE(7). Surprisingly, guanethidine was found to deplete NE from the brain stem of the rabbit to a degree equal to that observed after AMPT (Table I) and in similar fashion did not depress hypothalamic 5HT concentrations. With this degree of NE depletion in the brain, it is assumed that peripheral adrenergic neuron blockade has also resulted from guanethidine administration. Fig. 3 demonstrates that guanethidine pretreatment does not modify endotoxin fever.

A number of other pharmacologic agents were tested in this system without clear-cut results. Pretreatment with 5-hydroxytryptophan (5HTP), designed to raise hypothalamic 5HT concentrations and to determine whether or not endotoxin fever would thereby be enhanced, most often produced spontaneous fever prior to endotoxin challenge. Similarly, administration of the monoamine oxidase inhibitor, beta-isopropyl-phenylhydrazine (BIP), often caused spontaneous fever. Administration of this agent in dosage predictably not associated with spontaneous fever did not enhance febrile responses to endotoxin. A series of experiments in which animals pretreated with BIP or saline were acutely challenged with intravenous reserpine were performed to test the hypothesis that acute release of 5HT from brain stem stores would cause fever. Reserpine often produced a slight fever and monoamine oxidase inhibition appeared at times to accentuate this, but the effects were inconstant. An attempt was made to block the action of 5HT selectively in the brain stem using methysergide. However, administration of this agent also was associated at times with hyperpyrexia prior to endotoxin challenge and meaningful data could not be obtained.

Discussion. Several lines of evidence implicate 5HT as a mediator of fever production by the brain. Because 5HT does not readily cross the blood-brain barrier, it is not feasible to increase brain 5HT by infusion of the amine itself(8,9). Administration of 5-HTP, the immediate metabolic precursor of 5HT, does produce an increase in brain 5HT concentration and is consistently pyrogenic (10). When a compound inhibiting the degradation of 5HT to 5-hydroxyindoleacetic acid by monoamine oxidase is administered together with 5HTP, profound and often fatal febrile responses are seen. This enhancement of 5HTP fever has been employed as a phar-

macologic tool to assess the activity of various inhibitors of monoamine oxidase(11).

The observations of Hodge and associates offer further support of the role of 5HT in fevers(12). They demonstrated that the hyperpyrexia observed in guinea pigs following administration of tryptophan and a monoamine oxidase inhibitor was blocked by pretreatment with aromatic L-amino acid decarboxylase inhibitor (RO-4-4602). Pretreatment with the inhibitor also prevented the expected rise in brain 5HT after tryptophan. Because the fever produced by the amino acid precursors of 5HT may be enhanced by monoamine oxidase inhibition and blocked by decarboxylase inhibition, it appears that 5HT is the mediator of the fever.

Feldberg and Myers have demonstrated that infusion of 5HT directly into the ventricular system of cats elevates body temperature and that NE infused in similar fashion then lowers temperature(1,2). The action of intraventricular NE in lowering body temperature was confined to the 5HT-induced hyperpyrexic state; no lowering of normal body temperature was observed. On the basis of these experiments, Feldberg has proposed a general scheme of temperature regulation viewing 5HT and NE as opposing neurohumors acting at hypothalamic temperature-regulatory loci. Cooper and associates, using rabbits rather than cats, were unable to confirm the findings of Feldberg and Myers, however(13).

The present studies demonstrate that inhibition of endotoxin fever by reserpine is associated with depletion of brain stem 5HT and NE. Selective depletion of NE to a comparable degree by either AMPT or guanethidine treatment does not inhibit the febrile response. These results indicate that the effects of reserpine on endotoxin fever are not related to changes in CNS NE concentration. Whereas these studies do not exclude other possible pharmacologic actions of reserpine, they are consistent with the hypothesis that the important change induced by reserpine is the observed depletion of 5HT. Since guanethidine, in doses sufficient to deplete brain stem NE, does not inhibit fever, it is also unlikely that peripheral adrenergic neuron blockade accounts for the reserpine effect.

Summary. Reserpine in dosage sufficient to lower brain stem 5-hydroxytryptamine and norepinephrine suppresses endotoxin fever in rabbits. Pretreatment of rabbits with agents depleting only brain stem norepinephrine but not 5-hydroxytryptamine does not suppress febrile responses to endotoxin. An unexpected finding was the depletion of norepinephrine from the brain stem by guanethidine.

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