(at 35–40 days) than in the liver (at 50–55 days). The addition of adrenal preparations from immature animals did not inhibit the metabolism *in vitro* of DMBA by mature rat adrenals and, in all three cases, 7-OHM-12-MBA was formed by liver microsomes.

It is, of course, always dangerous to extend results *in vitro* to the situation in the intact animal. Nevertheless, it does appear that neither the metabolism of DMBA by the liver nor the presence of an adrenal gland actively producing corticosterone can account for the adrenocorticolytic effect of DMBA in the adult rat and yet not in the infant rat or mouse. Some other factor, such as the sensitivity of rat adrenal lysosomes to 7-OHM-12-MBA[9] or the susceptibility of the adrenal vascular bed in this species,[2] may have to be invoked to explain the highly selective action of this polycyclic hydrocarbon.

The findings of Huggins *et al.*[10] that marked depression in DNA synthesis in rat tissues and a great increase in the formation of liver menadione reductase were produced by doses of DMBA which, on a body weight basis, had little effect in mice, may also be important.

Cancer Research Centre,
University of British Columbia,
Vancouver, Canada

P. H. JELLINCK
SHEILA COLES
MAUREEN GARLAND

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Hyperthermia and elevated brain 5-hydroxytryptamine of rabbits in response to tryptophan and 5-hydroxytryptophan infusion*

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IT IS KNOWN that administration of 5-hydroxytryptophan (5-HTP) or L-tryptophan (L-Try) will cause hyperthermia in mammals and birds. This effect is presumed to be due to 5-hydroxytryptamine (5-HT). Feldberg and Myers[1, 2] have shown hyperthermia with 5-HT injected into the cerebral ventricals of cats in the area of the hypothalamus through an indwelling cannula.

It is the purpose of this investigation to compare various brain levels of 5-HT after infusion of 5-HTP and L-Try in monoamine oxidase-inhibited rats, and further to attempt to relate these changes to a physiological parameter, body temperature. The areas of the central nervous system examined are the hypothalamus, pons-medulla region, thalamus, and white and gray cortical matter. The peripheral organs examined are the liver and stomach.

MATERIALS AND METHODS

Male New Zealand white rabbits (1·6–2·1 kg) were used. Under light thiopental anesthesia, supplemented with procaine locally, a Teflon cannula was inserted retrograde into the external carotid

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artery with the tip of the cannula at the bifurcation. The opposite common carotid was ligated to insure circulation of the infused material to both sides of the brain.

The rabbits were allowed to recover overnight with water but no food. The following morning flexible rectal probes were inserted and taped to the tail. Temperatures were recorded at 15-min intervals with a Tele-thermometer (models 43TF and 4002, Yellow Springs Instrument Co., Inc.).

After stabilization of the temperature, *trans*-2-phenylcyclopropylamine (PCP, 10 mg/kg) was injected intraperitoneally. One half hr later the animals were infused with (5-HTP 0·55 mg/ml), L-Try (10·2 mg/ml), or normal saline at the rate of 0·051 ml/min by means of an infusion withdrawal pump (model 600–900, Harvard Apparatus Co.). The rate and concentrations were chosen to give the same concentrations of 5-HT in the hypothalamus for both 5-HTP- and L-Try-infused animals. At the end of a 2-hr infusion period, the animals were sacrificed by stunning and exsanguination. The brain, stomach, and liver were removed and frozen until determinations were made.

Spectrophotofluorometric determination of 5-HT was performed by the method of Udenfriend *et al.*[3] on a fluoromicrophotometer (model 4-7102, American Instrument Co., Inc.).

RESULTS

Infusion of 5-HTP and L-Try give similar behavioral effects. These effects are essentially the same as those observed by Bogdanski *et al.*[4] and are summarized as follows: excitement followed by depression, mydriasis, and increased salivary and nasal secretions, increased respiratory rate and elevated temperature when compared to controls infused with normal saline at the same rate.

There is an increase in rectal temperature beginning 15–30 min after start of the infusion of 5-HTP and L-Try (Fig. 1). At 1½ and 2 hr, there is a significant difference between temperatures of animals with 5-HTP and L-Try. 5-HTP causes a greater rise in temperature than L-Try.

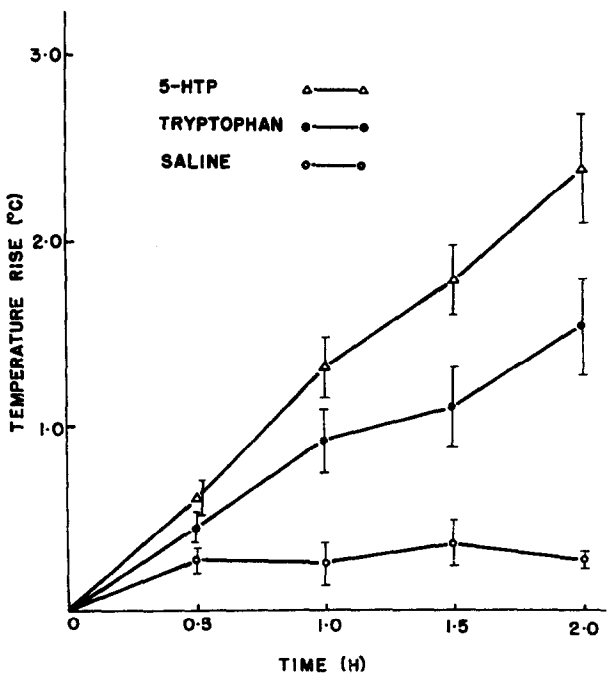

FIG. 1. This illustrates the effect on the rectal temperature of rabbits from the infusion of L-tryptophan (10·2 mg/ml, 0·051 ml/min), 5-hydroxytryptophan (0·55 mg/ml, 0·051 ml/min), or saline (0·051 ml/min) after a ½-hr pretreatment with *trans*-2-phenylcyclopropylamine (10 mg/kg, i.p.) When tryptophan is compared with control, the temperatures differed at 1½ and 2 hr ($P < 0·05$). When 5-HTP is compared with control, all readings are significantly different ($P < 0·01$). When 5-HTP and tryptophan are compared, they are significantly different at 1½ and 2 hr ($P < 0·05$).

The 5-HT levels are increased with both precursors in the following central structures: hypthalamus, thalamus, pons-medulla region, white and gray cortical matter (Table 1). There are no significant differences in stomach and liver.

TABLE 1. 5-HYDROXYTRYPTAMINE LEVELS IN RABBIT BRAIN (μg/g TISSUE) AND BODY TEMPERATURE (C°)*

Treatment	Pons-medulla	Hypo-thalamus	Thalamus	Cortical white matter	Cortical gray matter	Relative temperature
PCP + (4) Saline	0·58 ±0·07	1·38 ±0·13	0·71 ±0·08	0·21 ±0·06	0·35 ±0·03	40·80 ± 0·03
PCP + (6) Tryptophan	1·06 ±0·12‡	2·06 ±0·26†	1·02 ±0·10†	0·37 ±0·05†	0·49 ±0·05†	42·00 ± 0·25
PCP + (6) 5-HTP	1·05 ±0·09‡	1·94 ±0·26	1·47 ±0·20‡§	0·79 ±0·13‡‖	0·58 ±0·06‡	43·20 ± 0·29

* The effect on the 5-hydroxytryptamine levels in rabbit brain (μg/g tissues) from the infusion of saline, L-tryptophan (10·2 mg/ml) or 5-hydroxytryptophan (0·55 ml/min) at a rate of 0·051 ml/min after MAO inhibition by *trans*-2-phenylcyclopropylamine. Animals were infused for 2 hr via the internal carotid 30 min after treatment with the MAO inhibitor (10 mg/kg). Numbers in parentheses indicate the number of animals in each group. 5-HTP and L-Try animals were compared to control († $P < 0·05$, ‡ $P < 0·01$). Comparisons were made between 5-HTP and L-Try (§ $P < 0·05$, ‖ $P < 0·01$). Relative temperature is also given; see Fig. 1 for significance.

The 5-HT levels differ significantly between 5-HTP- and L-Try-infused animals in two central structures, the thalamus and white cortical matter. The 5-HT levels are higher with 5-HTP in these two structures.

DISCUSSION

When 5-HTP and L-Try, the precursors of 5-HT, are given in such a manner that the levels in the hypothalamus are the same, the temperatures of the two groups differ significantly. The thalamus and white cortical matter have significantly higher levels of 5-HT after the infusion of 5-HTP than after L-Try. Two important implications are evident. First, if the regulation of body temperature is dependent upon 5-HT in the hypothalamus, it appears that this control can be influenced by changes of 5-HT levels in other areas of the central nervous system; second, 5-HT is found in greater amounts in more areas of the central nervous system with 5-HTP as a precursor than with L-Try.

5-HT formation from L-Try is highest in the hypothalamus, pons-medulla, and thalamus. This correlates well with the experiments *in vitro* of Graham-Smith[5] and Green and Sawyer,[6] which show that tryptophan hydroxylase activity is highest in hypothalamus and thalamus. Our experiments indicate either some tryptophan hydroxylase activity in the white and gray cortical matter or transport of hydroxylated metabolite to these areas.

It is shown that 5-HTP and L-Try elevate temperature in rabbits. The degree of temperature rise is dependent upon which precursor is given. The increase is significantly higher with 5-HTP than when L-Try is administered. Levels of 5-HT are significantly higher in the cortical white matter and thalamus when 5-HTP is infused compared to animals infused with L-Try. 5-HT levels do not differ between 5-HTP- and L-Try-infused animals in the pons-medulla region, hypothalamus or gray matter. With both of these precursors, there are increases in all of these central structures when compared to saline-infused animals.

Department of Pharmacology,
University of Washington,
School of Medicine,
Seattle, Washington, U.S.A.

LAVERN J. WEBER
L. ALWINN ANGELL

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Stress and the maintenance of nicotinamide-induced hepatic nicotinamide adenine dinucleotide elevation by chlorpromazine

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IT IS KNOWN that the administration of nicotinamide, a precursor of the pyridine moiety of NAD, will result in a transient elevation of hepatic NAD levels in the mouse and rat. Burton *et al.*[1] observed that the administration of sedative doses of chlorpromazine or reserpine to mice 4 hr prior to the administration of nicotinamide results in prolongation of the nicotinamide-induced elevation of hepatic NAD. The administration of chlorpromazine or reserpine alone did not produce a detectable change in hepatic NAD levels compared to saline-treated controls. Similar results have been obtained with derivatives of chlorpromazine and reserpine such as deserpidine and promazine.[1, 2] A positive correlation has been observed between the tranquilizing activity of these compounds and their ability to prolong nicotinamide-induced hepatic NAD elevation. Burton *et al.*[2] have also shown that non-tranquilizing sedatives such as meprobamate, phenobarbital, and ethanol are unable to maintain elevated hepatic NAD levels even though doses were employed which maintained the mice in a comatose state.

It has been suggested that the relationship between reserpine or chlorpromazine and hepatic NAD levels after nicotinamide administration may be implicated in the mechanism of tranquilization or, at least, may be a reflection of a common biochemical action, since nontranquilizing congeners of these drugs are without effect in modifying NAD levels.

The pituitary–adrenal axis has also been demonstrated to exert an influence upon the level of hepatic NAD; the most pronounced effects are observed after the administration of nicotinamide. The administration of nicotinamide to hypophysectomized or adrenalectomized rats results in a much greater and more prolonged elevation in hepatic NAD levels than in intact controls.[3] Since both chlorpromazine and reserpine have been shown to produce adrenal ascorbic acid depletion and plasma corticosterone elevation,[4] it was decided to investigate the effect of other treatments which influence the pituitary–adrenal axis upon nicotinamide-induced hepatic NAD elevation.

METHODS

Male Holtzman rats (120–220 g) were maintained in communal cages housing 12–15 animals with free access to laboratory chow and water. After receipt from the supplier, rats were acclimatized for at least 1 week prior to use as experimental subjects. Hepatic NAD levels were determined spectrophotometrically by the procedure described by Ciotti and Kaplan[5] for the Racker alcohol dehydrogenase assay. This procedure is specific for determination of oxidized NAD. Adrenal ascorbic acid levels were determined spectrophotometrically by an adaptation[6] of the method described by Sullivan and Clarke.[7]

All drugs were administered 4 hr prior to nicotinamide (500 mg/kg) and were dissolved in distilled water in sufficient concentration so that injection of 1 ml solution/kg body wt. provided the desired dosage. Nicotinamide, chlorpromazine hydrochloride (25 mg/kg), and chlorpromazine sulfoxide hydrochloride (25 mg/kg) were injected i.p. ACTH (250 mU/rat) was administered i.v. via the