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Effect of Chronic Dietary Treatment With l-Tryptophan on Spontaneous Salt Appetite of Rats[1]

MELVIN J. FREGLY,[2] NEIL E. ROWLAND AND COLIN SUMNERS

Departments of Physiology and Psychology, Colleges of Medicine and Liberal Arts and Sciences
University of Florida, Gainesville, FL 32610

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FREGLY, M. J., N. E. ROWLAND AND C. SUMNERS. *Effect of chronic dietary treatment with l-tryptophan on spontaneous salt appetite of rats*. PHARMACOL BIOCHEM BEHAV 33(2) 401–406, 1989.—Chronic dietary treatment with l-tryptophan (2.5 and 5.0% in food) reduced the exaggerated spontaneous NaCl intake induced by deoxycorticosterone acetate (DOCA) in rats. In the absence of DOCA, chronic (4-week) dietary treatment with l-tryptophan (5.0% in food) failed to affect significantly spontaneous NaCl intake. In addition, treatment with tryptophan in the absence of DOCA failed to affect significantly systolic blood pressure, plasma renin activity, plasma aldosterone concentration, and the weights of the heart, kidneys, adrenals, and thyroid gland. It also failed to affect the specific binding of [^{125}I]-Ang II to neuronal membranes isolated from the diencephalon of the rats. However, the contents of both serotonin and 5-hydroxyindoleacetic acid in the lower brain stem were increased significantly by chronic treatment with tryptophan. These results suggest that the effect of chronic treatment with tryptophan to reduce the salt appetite of DOCA-treated rats is a specific effect of this amino acid under these conditions and may be related to the ability of tryptophan to prevent the upregulation of specific Ang II receptors induced by DOCA.

Salt appetite l-Tryptophan Deoxycorticosterone acetate Angiotensin II Aldosterone Plasma renin activity

EARLIER studies from this laboratory reported that the exaggerated intake of NaCl solution induced in rats by chronic treatment with deoxycorticosterone acetate (DOCA) was reduced by chronic dietary treatment with l-tryptophan (6,9). In these studies the rats were unilaterally nephrectomized and were given *only* 0.15 M NaCl solution to drink. A possibility existed that the reduction in intake of NaCl solution under these conditions might be due to a change in palatability induced by tryptophan. To assess this possibility more fully, the effect of chronic treatment with tryptophan on *spontaneous* intakes of water and 0.15 M NaCl solution was tested in a two-bottle choice paradigm using, in separate experiments, both unilaterally nephrectomized, DOCA-treated, and intact, untreated rats. Additional studies were also carried out in intact rats to assess the effect of treatment on a number of other parameters that had been measured previously when tryptophan was administered to DOCA-treated rats, including food intake, urine output, plasma renin activity, plasma aldosterone concentration, and systolic blood pressure. The results of administration of tryptophan alone are compared and contrasted with those observed when tryptophan was administered in combination with DOCA.

METHOD

Experiment 1: Effect of Chronic Dietary Treatment With Tryptophan on Spontaneous Salt Appetite of Unilaterally-Nephrectomized, DOCA-Treated Rats

Twenty-four female rats of the Blue Spruce Farms (Sprague-Dawley) strain weighing initially from 180 to 220 g were used. The rats were provided with tap water and finely powdered Purina Laboratory Chow (No. 5001) ad lib. Infant nursing bottles with cast aluminum spouts were used as fluid containers (14). Food containers were spill-resistant and have been described in detail (4). The vivarium was maintained at 26 ± 1°C and illuminated from 7 a.m. to 7 p.m. During this period, the rats were maintained 3 per cage.

The rats were divided randomly into four equal groups. Unilateral (left) nephrectomy was then carried out in all animals while they were anesthetized with pentobarbital (40 mg/kg, IP). At this time, two preweighed 25-mm long Silastic tubes (No. 602-265), filled with DOCA (Sigma Chemical Co., No. D-7000) and sealed with Silastic cement, were implanted SC between the shoulder blades of three of the four groups. The remaining group was implanted SC with an empty Silastic tube. At the end of the experiment, the tubes were removed, dried for 72 hours in a desiccator, and weighed on an analytical balance. Based on the change in weight of the tubes and the average weight of the

[1]Supported by grant HL25459 from the National Heart, Lung, and Blood Institute.
[2]Request for reprints should be addressed to Dr. Melvin J. Fregly, Department of Physiology, Box J274, University of Florida, College of Medicine, Gainesville, FL 32610.

animals during the time the tubes were implanted, an average of 801 ± 46 μg DOCA/kg/day (249 ± 31 μg/day) were released from the tubes.

Immediately after unilateral nephrectomy and implantation of Silastic tubes, two of the three DOCA-treated groups received l-tryptophan (Sigma Chemical Co., No. T0254) mixed into their finely powdered Purina Laboratory Chow at a concentration of 25 and 50 g/kg of food, respectively. The tryptophan and a small amount of food were first triturated with a mortar and pestle. This was then added to a kg of food and mixed in a tumbling mixer at 4 rpm for 1 hr. The remaining DOCA-treated group and the control group received the same food without tryptophan. All rats were allowed only 0.15 M NaCl solution to drink for 11 weeks.

During the twelfth week, the rats were placed individually into metabolic cages and given a choice between distilled water and 0.15 M NaCl solution to drink. Spontaneous intakes of food, water, and NaCl solution were measured daily for 4 days.

Experiment 2: Some Effects of Chronic Dietary Treatment With Tryptophan in Intact Rats

Twelve female rats of the Sprague-Dawley strain weighing 180–220 g were used. They were divided at random into two equal groups. Group 1 received finely powdered Purina Laboratory Chow (No. 5001) into which was mixed 50 g of l-tryptophan/kg of food. Food and fluid containers, as well as other conditions of maintenance, were the same as those used in Experiment 1.

During the second week on the diet, the drinking response to an acute SC injection of angiotensin II (200 μg/kg) was measured. On the day of the experiment (9:00 a.m.) the rats were weighed, injected with angiotensin II (Ang II, Sigma Chemical Co., No. 9525) and placed in individual cages. Each rat was then provided with a preweighed bottle of tap water (26°C). No food was available to the rats during this study. Fluid intakes were then measured at 0.5, 1.0 and 2.0 hr after injection of Ang II. Urine output was also measured at these times. The concentrations of sodium and potassium in the pooled 2-hr urine sample were measured by flame photometry using lithium as the internal standard. The rats were returned to their stock cages at the end of this experiment.

TABLE 1

EFFECT OF CHRONIC DIETARY TREATMENT WITH TRYPTOPHAN ON DAILY INTAKE OF FOOD, WATER AND 0.15 M NaCl SOLUTION BY DOCA-TREATED RATS*

Treatment	No. of Rats	Mean Body Wt. (g)	Mean Daily Intake (g or ml/100 g b.wt.) of: Food	Water	NaCl	Urine Output (ml/100 g b.wt.)
Control	6	312 ±6[a]	4.7 ±0.2	5.2 ±1.0	12.3 ±2.4	13.2 ±1.3
DOCA	6	328 ±9	5.6 ±0.5	8.1 ±2.4	26.9 ±4.5[b]	28.3 ±4.9[b]
DOCA + 2.5% Tryptophan	6	297 ±11	4.5 ±0.1	5.2 ±0.9	22.0 ±8.9	22.8 ±8.2
DOCA + 5.0% Tryptophan	6	302 ±13	5.3 ±0.6	6.7 ±1.3	18.1 ±6.0	18.4 ±4.3

*Performed during week 12 of the study; data represent means of four days of intake.
[a]One standard error of mean.
[b]Significantly different from Control ($p<0.01$).

During the fourth week of treatment with tryptophan, the spontaneous appetite for NaCl solution was measured in all rats. The animals were caged individually and provided with their usual food, but given a choice of drinking fluid between two bottles, one containing distilled water and the other 0.15 M NaCl solution. Intakes and urine outputs of each rat were measured daily for four days. The urines collected daily were analyzed for their sodium and potassium concentrations by flame photometry and for their concentrations of epinephrine, norepinephrine, and dopamine by

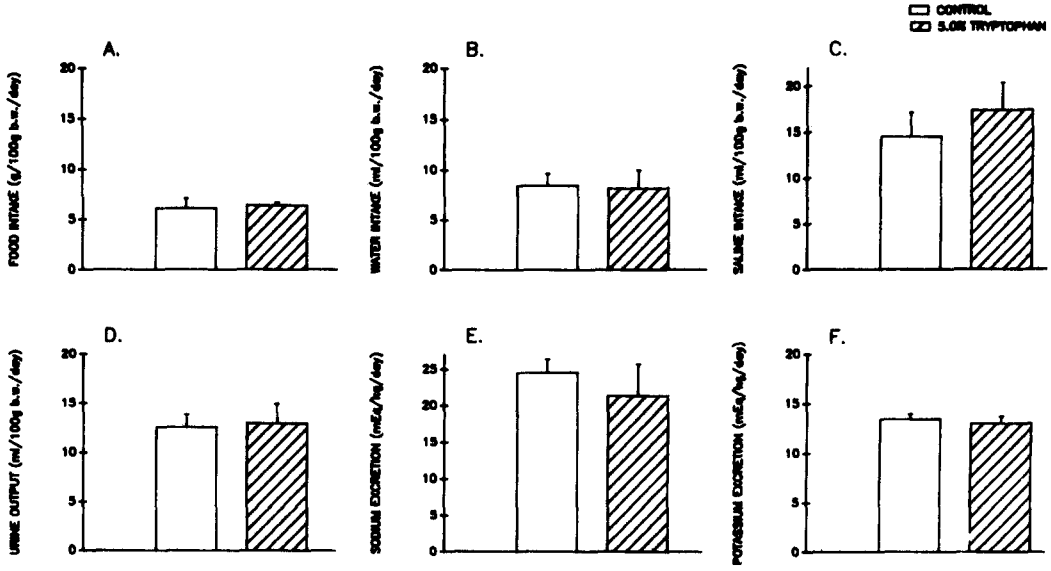

FIG. 1. This figure illustrates the effect of chronic administration of tryptophan (5% in diet) on spontaneous intake of isotonic saline (A), water (B), food (C), urine output (D), urinary sodium excretion (E), and urinary potassium excretion (F). The means of four days of measurement in individual rats are shown. Control and treated groups are designated in the figure. One standard error is set off at each mean. There were no significant differences between groups.

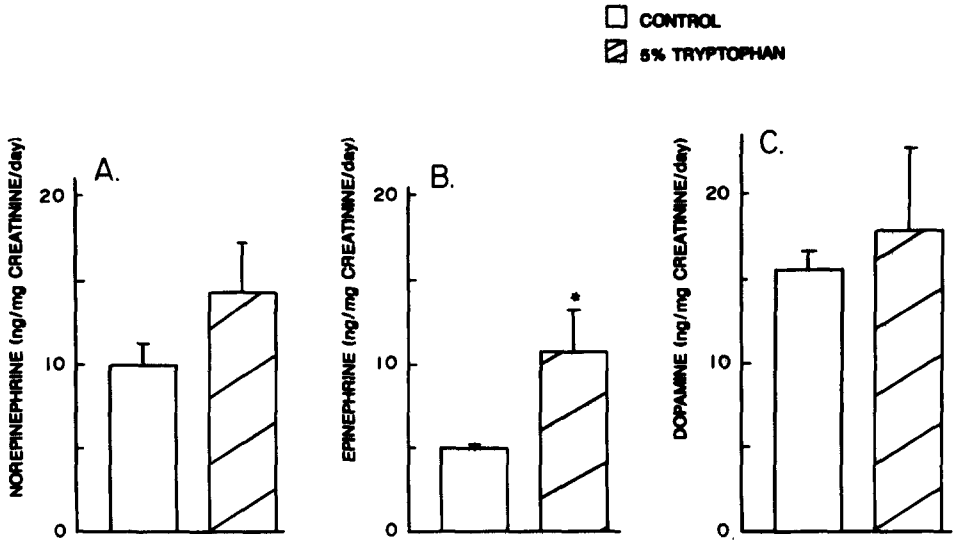

FIG. 2. This figure illustrates the mean daily urinary output of norepinephrine (A), epinephrine (B), and dopamine (C) during the same four-day period shown in Fig. 1. Control and treated (5% tryptophan in diet) groups are designated in the figure. One standard error is set off at each mean. *$p<0.05$ compared to the control group.

high pressure liquid chromatography with electrochemical detection (1,13).

During the fifth week of the study, the rats were surgically prepared with an indwelling intracerebroventricular (ICV) cannula. Using Equithesin (1.5 ml/kg, IP) for anesthesia and a Kopf stereotaxic instrument, a 23-gauge stainless steel tube (11 mm long) with a wire obturator was implanted to end at the dorsal part of the right lateral cerebral ventricle (coordinates: 3.5 mm ventral, 1.5 mm lateral to bregma). The cannula was secured with skull screws and dental acrylic cement, and a postoperative recovery period of three days was allowed, after which experiments were begun. On the day of the study (9:00 a.m.) each rat was weighed and Ang II (10 ng in 2 μl of sterile isotonic saline) was administered ICV. The rats were then placed in individual cages without food and given a preweighed bottle of distilled water. Water intake and urine output of each rat were measured at 1.0 and 2.0 hours thereafter. The urinary sodium and potassium concentrations of the pooled 2.0-hour urine sample for each rat were measured, and sodium and potassium outputs determined.

At the beginning of the sixth week of the study, the systolic blood pressure of each rat was measured twice by means of the tail cuff technique (5). At the end of the week, the rats were killed by guillotine decapitation and blood collected from the trunk for measurement of plasma renin activity (PRA, in EDTA, New England Nuclear, Boston, MA) and plasma aldosterone (Diagnostics Products, Inc., Los Angeles, CA) concentration by radioimmunoassay. At death, the heart, kidneys, adrenals, and thyroid gland were removed, cleaned of extraneous tissue, and weighed on a torsion balance. In addition, the brain was removed and used in a receptor binding assay for Ang II. The binding technique used was modified from that of Sirett *et al.* (16) and has been described in detail in earlier publications from this laboratory (11,17). In brief, the brain was cut anterior to the preoptic region and at the level of the mammillary bodies to represent the anterior and posterior limits of the diencephalic block. The lateral limits included the edges of the lateral hypothalamus. The block of tissue (mean weight = 100 mg) included the thalamus, hypothalamus, and septum (HTS). Specific areas included the preoptic area, paraventricular nucleus, organum vasculosum of the lamina terminalis, subfornical organ, anterior and posterior nuclei, and dorsomedial and ventromedial nuclei. The diencephalic block was homogenized in a ground glass homogenizer in 20 volumes of ice-cold 0.9% saline, and the homogenate centrifuged at 600 × g and 4°C for 10 min to remove all blood vessels and connective tissue. The supernatants were then decanted into another tube on ice and then centrifuged at 50,000 × g and 4°C for 30 min. The pellet from this centrifugation, which contains the brain membranes, was used for the binding assay. Protein concentration of the brain particulate fraction was determined by the method of Lowry *et al.* (15), and binding data were expressed as fmol/mg protein.

The lower brainstem of each rat was weighed and homogenized in 1 ml of 0.1 M perchloric acid with glutathione (10 μg) and disodium EDTA (50 μg) added. The homogenate was centrifuged in a refrigerated centrifuge (3,000 × g); the supernatant was removed and filtered using Millipore filters (0.2 μM). The filtrate was assayed for norepinephrine, serotonin, and 5-hydroxyindole acetic acid directly by HPLC. Chromatographic conditions were similar to those used to analyze urinary catecholamines. All values were standardized to a unit of wet tissue weight.

Statistical analysis was carried out by means of Student's *t*-test. Significance was set at the 95% confidence limit.

RESULTS

Experiment 1

When uninephrectomized, DOCA-treated rats were offered a choice between water and 0.15 M NaCl solution to drink, they ingested more than twice the amount of NaCl solution as uninephrectomized controls (Table 1). There was no significant difference between the water intakes of the two groups. Chronic treatment with tryptophan at 2.5 and 5.0% mixed into the diet was accompanied by a stepwise reduction in intake of NaCl solution back toward the level of the controls. While NaCl intake of the group receiving 5.0% tryptophan did not differ significantly from

TABLE 2

EFFECT OF CHRONIC DIETARY TREATMENT WITH TRYPTOPHAN ON THE DIPSOGENIC RESPONSE TO ANGIOTENSIN II (200 μg/kg b.wt., SC)*

Treatment	No. of Rats	Mean Body Wt. (g)	Cum. Water Intake (ml/kg) During: 1 hr	2 hr	Cum. Urine Output (ml/kg) During: 1 hr	2 hr	Urinary Na Output (mEq/kg/ 2 hr)	Urinary K Output (mEq/kg/ 2 hr)
Control	6	248 ±5[a]	17.3 ±1.4	18.7 ±1.2	6.3 ±0.7	8.2 ±0.8	2.6 ±0.4	1.4 ±0.1
5.0% Trypt.	6	239 ±6	20.4 ±1.6	24.2 ±1.7[b]	6.1 ±1.9	12.1 ±1.7[b]	3.0 ±0.3	2.5 ±0.4[c]

*Performed during week 2 of study.
[a]One standard error of mean.
[b]Significantly different from Control ($p<0.05$).
[c]Significantly different from Control ($p<0.01$).

that of the control group, it also did not differ significantly from that of the DOCA-treated group. Urine output of the group receiving only DOCA was significantly ($p<0.01$) greater than that of the control group and was reduced progressively with increasing doses of tryptophan. These results indicate that chronic administration of tryptophan to DOCA-treated rats reduced their exaggerated intake of NaCl solution. Food intake and body weight were not affected significantly by treatment.

Experiment 2

In contrast to the results with DOCA-treated rats, chronic administration of tryptophan (5.0% in diet) alone had no significant effect either on intake of NaCl solution or water (Fig. 1B and C). Food intake and urine output were also unaffected by treatment (Fig. 1A and D), as were also the cases with sodium and potassium excretion (Fig. 1E and F).

The daily urinary output of norepinephrine and dopamine were unaffected by chronic treatment with tryptophan (Fig. 2A and C), but urinary output of epinephrine was nearly doubled (Fig. 2B).

Acute peripheral administrtion of Ang II (200 μg/kg, SC) increased water intake and urine output of the treated group during the second hour to a level significantly ($p<0.05$) above that of the control group (Table 2). Urinary potassium output of the treated group during the two hours was also increased significantly ($p<0.01$).

Although central administration of Ang II (10 ng/rat) induced a somewhat greater increase in water intake in the tryptophan-treated group, the increase was not significant (Table 3). Nor was there a significant effect of treatment on either urine output or urinary electrolyte output.

Systolic blood pressure, measured during the last week of the study, was not affected significantly by treatment (Table 4). At death, measurement of the weights of heart, kidneys, adrenals and thyroid gland failed to reveal any significant differences between groups (Table 4). There were also no significant differences in either plasma renin activity or plasma aldosterone concentration.

Analysis of the content of norepinephrine, serotonin, and 5-hydroxyindole acetic acid in the lower brainstem of treated and control rats revealed a significant ($p<0.01$) increase in serotonin and HIAA contents in the tryptophan-treated group (Table 5). Norepinephrine content of the mesencephlon was not affected significantly by treatment.

The specific binding of [^{125}I]-Ang II to brain membranes prepared from the diencephalon of the treated rats at 0.25 and 1.0

TABLE 3

EFFECT OF CHRONIC DIETARY TREATMENT WITH TRYPTOPHAN ON THE DIPSOGENIC RESPONSE TO ANGIOTENSIN II (10 ng IVT)*

Treatment	No. of Rats	Mean Body Wt. (g)	Cum. Water Intake (ml/kg) During: 1 hr	2 hr	Cum. Urine Output (ml/kg) During: 1 hr	2 hr	Urinary Na Output (mEq/kg/ 2 hr)	Urinary K Output (mEq/kg/ 2 hr)
Control	6	243 ±4[a]	33.5 ±8.3	36.9 ±8.4	5.7 ±2.6	24.2 ±7.0	0.64 ±0.30	0.72 ±0.11
5.0% Trypt.	6	234 ±7	39.5 ±3.0	41.9 ±3.3	2.8 ±1.3	22.5 ±3.0	0.30 ±0.09	0.51 ±0.08

*Performed during week 5 of study.
[a]One standard error of mean.

TABLE 4

EFFECT OF CHRONIC DIETARY TREATMENT WITH TRYPTOPHAN ON ORGAN WEIGHTS, PLASMA RENIN ACTIVITY, PLASMA ALDOSTERONE CONCENTRATION AND SYSTOLIC BLOOD PRESSURE OF RATS*

Treatment	Organ/Body Weight Ratios (mg/100 g b.wt.) of: Heart	Kidney	Adrenal	Thyroid	Plasma Aldosterone (pg/ml)	PRA (ng/ml/hr)	Systolic B.P. (mmHg)
Control	334.5 ±12.4[a]	681.6 ±38.1	31.3 ±1.0	6.9 ±0.5	129.2 ±42.4	2.9 ±0.6	95 ±5
5.0% Trypt.	319.5 ±6.2	653.6 ±11.6	31.7 ±1.6	7.9 ±0.3	91.4 ±24.0	2.8 ±0.5	101 ±5

*Performed during week 6 of study.
[a]One standard error of mean.

nM of Ang II was 17.2±0.5 and 72.5±3.1 fmol/mg protein, respectively. The specific binding for the control group was 14.7±1.6 and 65.1±5.2 fmol/mg protein at 0.25 and 1.0 nM of Ang II, respectively. There were no significant differences between groups.

DISCUSSION

The results of Experiment 1, carried out under conditions in which the rats were allowed choice between water and isotonic saline, show that chronic administration of tryptophan reduced the salt appetite of DOCA-treated rats. These results are similar to those reported earlier (6,9) in a paradigm in which the DOCA-treated rats were allowed *only* isotonic saline to drink. This effect of tryptophan in the presence of DOCA, was not observed in the absence of DOCA (Experiment 2). Thus, tryptophan alone does not appear to affect spontaneous intake of NaCl solution in a two-bottle choice paradigm. This suggests that the mechanism by which tryptophan affects the intake of NaCl solution in DOCA-treated rats is via a reversal of one or more physiological or biochemical changes induced by DOCA. A possible mechanism by which tryptophan could affect the salt appetite of DOCA-treated rats may involve central receptors for Ang II. Thus, the increased intake of NaCl solution by DOCA-treated rats was attributed to the upregulation of their central Ang II receptors (9). Further, chronic treatment with tryptophan was shown to return regulation of central Ang II receptors to the level of controls (9). Since both Ang II and aldosterone are important factors influencing spontaneous intake of NaCl in rats (10), prevention of the upregulation of central Ang II receptors by tryptophan could explain its inhibitory effect on the salt appetite of DOCA-treated rats.

In contrast to the effects of chronic treatment with tryptophan to reduce the spontaneous NaCl intake of DOCA-treated rats, the results of the present study also indicate that it had no effect on spontaneous intake of either NaCl solution or water in rats not treated with DOCA. Also in contrast with the results in DOCA-treated rats, tryptophan had no effect on the specific binding of Ang II to its receptors in the diencephalon. This suggests, but does not prove, that there is a relationship between the specific binding of Ang II to its receptors in the diencephalon and spontaneous intake of NaCl solution in rats.

The increased urinary output of epinephrine has been observed previously in both tryptophan- (7,9) and 5-hydroxytryptophan-treated rats (8). The significance of this observation is unclear, except to suggest that chronic treatment with tryptophan may increase the production of epinephrine by the adrenal medulla. Presumably, this could occur if tryptophan induced the enzyme, phenylethanolamine-n-methyl transferase in the adrenal medulla. This enzyme is responsible for converting norepinephrine to epinephrine. Whether the antihypertensive effect of tryptophan in DOCA-treated and renal hypertensive rats is associated with an increased production of epinephrine, and the decrease in peripheral vascular resistance associated with it, remains for further study (2). If this is the case, it is clear that normotensive rats appear to respond less well, with respect to blood pressure, than DOCA-treated, hypertensive rats. The mechanism is unclear and deserves further study.

The dipsogenic responsiveness to acute SC administration of Ang II in tryptophan-treated rats was greater than that of controls. Since the specific binding of Ang II to its receptors in the diencephalon was unchanged by treatment with tryptophan, it seems unlikely that an increased specific binding of Ang II to its receptors played a role. The mechanism(s) by which the increased responsiveness occurred is not revealed by these studies and remains to be elucidated. It is of interest in this regard that central administration of Ang II did not induce a greater dipsogenesis in tryptophan-treated rats. This suggests that a peripheral mechanism may play a role.

Although chronic treatment with tryptophan has been shown to exert antihypertensive effects on DOCA-induced, renal, and SHR hypertensions, it did not affect the systolic blood pressure of otherwise untreated rats (6, 7, 9, 12). This suggests that it acts *specifically* either to reduce blood pressure, or to prevent its elevation, in these types of experimentally-induced hypertensions.

TABLE 5

EFFECT OF CHRONIC DIETARY TREATMENT WITH TRYPTOPHAN ON NOREPINEPHRINE, SEROTONIN, AND 5-HYDROXYINDOLE ACETIC ACID CONTENTS OF THE LOWER BRAIN STEM OF RATS

Treatment	No. of Rats	Norepinephrine (ng/g)	Serotonin (ng/g)	HIAA (ng/g)
Control	6	461 ±33*	603 ±65	530 ±106
5.0% Trypt.	6	450 ±31	770 ±44[a]	1078 ±253[a]

*One standard error of mean.
[a]Significantly different from control ($p<0.01$).

Further the results suggest that the antihypertensive effect of tryptophan in DOCA-treated rats is specific since the blood pressure of normotensive rats was unaffected by treatment.

Chronic administration of tryptophan at the dose used had no effect on either food intake or body weight. This suggests that this dose of tryptophan was not toxic and was well tolerated by the rats. This was also attested to by the similar food intakes and body weights of the treated and control groups.

Analysis of the content of catecholamines in the lower brainstem of treated and control rats revealed that the tryptophan administered to the rats accessed the brain since the contents of serotonin and its metabolite, 5-hydroxyindole acetic acid, were increased significantly above the level of the control group. This observation suggests a greater turnover of serotonin in the mesencephalon of treated rats and verifies similar results of others (3).

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