Biochemical Pharmacology, Vol. 18, pp 2061–2067. Pergamon Press. 1969. Printed in Great Britain

5-HYDROXYTRYPTAMINE LEVELS IN PERIPHERAL ORGANS OF IMMUNOSYMPATHECTOMIZED RATS*†

GERDA I. KLINGMAN

Department of Biochemical Pharmacology, School of Pharmacy, State University of New York at Buffalo, Buffalo, N.Y. 14214, U.S.A.

(*Received* 9 *August* 1968; *accepted* 31 *January* 1969)

Abstract—Littermate Sprague–Dawley rats were immunosympathectomized by the daily injection of Nerve Growth Factor (NGF)-antiserum for 6 days after birth. The 5-hydroxytryptamine concentrations of peripheral tissues, spinal cord and brain stem (medulla, pons, mesencephalon and diencephalon) were measured spectrophotofluorometrically by the method of Bogdanski *et al.*‡ In immunosympathectomized rats the 5-hydroxytryptamine levels of the submaxillary glands, hearts and cecum were significantly increased. Statistically nonsignificant elevations were also noted in the lungs, upper intestinal tract, liver and uterus. The submaxillary glands of immunosympathectomized rats were significantly smaller than those of control littermates. The administration of JB-516 (Catron, β-phenylisopropylhydrazine) or 5-hydroxytryptophan did not present evidence to explain the elevated 5-hydroxytryptamine levels in some of the peripheral tissues from immunosympathectomized rats.

It is not known whether the rise of the 5-hydroxytryptamine levels in some peripheral tissues from immunosympathectomized rats was a direct result of the NGF-antiserum administration or whether it was due to the fact that these tissues are largely devoid of sympathetic innervation. The most marked and consistent elevation of 5-hydroxytryptamine occurred in those tissues (submaxillary glands and heart) which are nearly completely sympathectomized by the NGF-antiserum administration.

THE ACTIVITIES of dopa-decarboxylase, monoamine oxidase and catechol-*O*-methyltransferase in immunosympathectomized rats have been studied in this laboratory.[1–3] Some changes in the activites of these enzymes have been noted after immunosympathectomy. Since two of the three enzymes of catecholamine metabolism are probably also involved in the metabolism of 5 hydroxytryptamine, the concentration of this amine in peripheral organs, brain stem§ and spinal cord from immunosympathectomized rats has been investigated, and the results of this study are reported.

METHODS

Sprague–Dawley rats of both sexes, born and raised in this laboratory, were used in this investigation. Each litter of newborn animals was divided into two groups; controls and those immunosympathectomized by the subcutaneous daily injection of 300 units of Nerve Growth Factor (NGF)-antiserum/g for 6 days; the first dose

* Supported by Grant AM-06594 from the National Institute of Arthritis and Metabolic Diseases of the United States Public Health Service.

† The Nerve Growth Factor Antiserum was kindly supplied by Dr. R. K. Richards, formerly of Abbott Laboratories, Inc., North Chicago, Ill.

‡ F. Bogdanski, A. Pletcher, B. B. Brodie and S. Udenfriend, *J. Pharmac.* **117**, 82 (1956).

§ Medulla, pons, mesencephalon and diencephalon.

was given 5–8 hr after birth. This treatment resulted in 85–90 per cent destruction of the peripheral sympathetic nervous system, as assessed by total cell counts conducted on the superior cervical and stellate ganglia.[4–6]

Antiserum-treated rats were killed when 2–3 months old or when 7–9 months old by decapitation while anesthetized with sodium pentobarbital. Each immunosympathectomized rat was paired with a normal littermate of the same sex and 5-hydroxytryptamine levels were determined for peripheral organs, brain stem (medulla, pons, mesencephalon and diencephalon) and for the spinal cord.

Normal and antiserum-treated rats were treated with the monoamine oxidase inhibitor β-phenylisopropylhydrazine (Catron, JB-516),§ 10 mg/kg intraperitoneally (i.p.) 16–18 hr before sacrifice and the 5-hydroxytryptamine levels were determined in the tissues described above.

The effect of 5-hydroxytryptophan (150 mg/kg i.p. 3 hr before sacrifice) on 5-hydroxytryptamine levels in normal and antiserum-treated rats was also studied.

The method of Bogdanski *et al.*[7] was used to extract and determine the tissue 5-hydroxytryptamine concentrations spectrophotofluorometrically. For the 5-hydroxytryptophan-treated group, repeated washes of the *n*-butanol extract with borate buffer insured the removal of 5-hydroxytryptophan before the spectrophotofluorometric analyses of 5-hydroxytryptamine.

RESULTS

The similarity of the 5-hydroxytryptamine concentrations in tissues from rats of the two periods (2–3 and 7–9 months after immunosympathectomy) permitted pooling of data. In partially immunosympathectomized rats the 5-hydroxytryptamine concentrations of the heart, submaxillary glands and cecum were significantly increased. The mean 5-hydroxytryptamine levels of several other tissues (e.g. lungs,

TABLE 1. 5-HYDROXYTRYPTAMINE CONCENTRATIONS OF PERIPHERAL ORGANS, SPINAL CORD AND BRAIN STEM* FROM CONTROL AND IMMUNOSYMPATHECTOMIZED RATS

Tissue	No. of pairs	5-Hydroxytryptamine (μg/g wet weight ± standard error)	
		Control	Immunosympathectomy
Submaxillary glands	17	1·29 ± 0·13	2·51 ± 0·29†
Heart	16	0·26 ± 0·03	0·34 ± 0·03‡
Uterus	5	0·51 ± 0·02	0·81 ± 0·23
Spleen	16	3·63 ± 0·39	2·82 ± 0·20
Lungs	15	2·89 ± 0·48	3·60 ± 0·51
Kidneys	11	0·31 ± 0·03	0·29 ± 0·03
Liver	15	0·57 ± 0·08	0·63 ± 0·05
Small intestine, prox.	14	2·30 ± 0·22	2·93 ± 0·28
Small intestine, dist.	9	2·46 ± 0·35	2·77 ± 0·47
Cecum	7	3·39 ± 0·55	5·41 ± 0·66‡
Stomach	8	2·65 ± 0·23	2·62 ± 0·43
Brain stem*	5	0·40 ± 0·02	0·33 ± 0·02
Spinal cord	10	0·33 ± 0·05	0·40 ± 0·06

* Medulla, pons, mesencephalon and diencephalon.
† $P < 0.001$.
‡ $P < 0.05$.

§ The author wishes to thank Lakeside Laboratories for the generous supply of Catron

small intestine and uterus) were also elevated, while the concentration in the spleen was decreased (Table 1). These changes, however, were statistically nonsignificant ($P > 0{\cdot}05$). The total 5-hydroxytryptamine content of the submaxillary glands from antiserum-treated rats was significantly increased ($P < 0{\cdot}05$), while the changes of the total 5-hydroxytryptamine content of the heart, spleen and uterus were not statistically significant (Table 2).

TABLE 2. TOTAL 5-HYDROXYTRYPTAMINE CONTENT OF SOME PERIPHERAL TISSUES FROM CONTROL AND IMMUNOSYMPATHECTOMIZED RATS

Tissue	No. of pairs	Total 5-hydroxytryptamine content (μg/tissue $\pm$ standard error)	
		Control	Immunosympathectomy
Heart	16	$0{\cdot}34 \pm 0{\cdot}04$	$0{\cdot}41 \pm 0{\cdot}04$
Spleen	16	$2{\cdot}37 \pm 0{\cdot}20$	$2{\cdot}62 \pm 0{\cdot}29$
Submaxillary glands	17	$0{\cdot}85 \pm 0{\cdot}09$	$1{\cdot}19 \pm 0{\cdot}14$*
Uterus	5	$0{\cdot}40 \pm 0{\cdot}08$	$0{\cdot}33 \pm 0{\cdot}09$

* $P < 0{\cdot}05$.

A disparity of the body weights and some of the organ weights was noted between control and immunosympathectomized littermate rats (Table 3). Immunosympathectomized male rats were usually lighter than their control littermates. The submaxillary glands of both male and female rats were significantly smaller after immunosympathectomy. The ratios of the submaxillary glands to the body weights (mg/g) were also significantly smaller in immunosympathectomized rats (Table 3). However, ratios of the dry weight/wet weight and protein concentration/wet weight were not altered by immunosympathectomy in any of the peripheral tissues except the spleen. In the latter tissue the protein concentration per g of wet tissue was significantly increased in immunosympathectomized rats, 211 ± 9 mg/g in treated rats and 180 ± 8 mg/g in control rats ($P < 0{\cdot}02$; $n = 14$ in each group). Due to the absence of postganglionic sympathetic fibers in the capsule of the spleen of immunosympathectomized rats, the spleens did not contract during sacrifice. Consequently, the wet weights of spleens of immunosympathectomized rats were significantly greater than those of control animals (Table 3).

After the administration of β-phenylisopropylhydrazine (JB-516) or 5-hydroxytryptophan the per cent increases of 5-hydroxytryptamine were of similar magnitude in tissues from immunosympathectomized rats and their control littermates (Tables 4 and 5).

DISCUSSION

During the past 3 yr several publications dealing with the intestinal 5-hydroxytryptamine content of immunosympathectomized rats and mice have appeared in the literature. Hamberger *et al.*[9] suggested that the apparent increase of the 5-hydroxytryptamine fluorescence of the intestine from immunosympathectomized rats may have been due to the greater number of mast cells. Iversen *et al.*[10] reported elevated intestinal 5-hydroxytryptamine levels in immunosympathectomized rats but offered

TABLE 3. BODY AND TISSUE WEIGHTS OF CONTROL (C) AND IMMUNOSYMPATHECTOMIZED (IM) RATS

	Male rats			Female rats			Male and female rats			Male and female rats Catron-treated		
	n*	C	IM	n*	C	IM	n*	C	IM	n*	C†	IM†
Body weight (g)‡	12	527 ±16	465§ ±12	5	299 ±17	293 ±20	17	460 ±29	415 ±22	8	338 ±19	345 ±25
Submaxillary glands (mg)‡	12	713 ±29	506‖ ±21	5	529 ±31	393¶ ±48	17	659 ±30	473‖ ±23	8	535 ±51	437 ±32
Ratio of submaxillary glands/body weight (mg/g)‡	12	1·37 ±0·07	1·09§ ±0·05	5	1·77 ±0·03	1·34¶ ±0·16	17	1·49 ±0·07	1·17§ ±0·06	8	1·58 ±0·12	1·27 ±0·04
Spleen (mg)‡							16	790 ±47	1101§ ±90	8	626 ±45	927 ±56
Uterus (mg)‡				5	788 ±182	448 ±113				4	580 ±156	580 ±125
Heart (mg)‡							16	1304 ±71	1187 ±63	8	1098 ±83	1106 ±99

* Number of pairs.
† Catron, JB-516, β-phenylisopropylhydrazine; i.p., 10 mg/kg, 16–18 hr prior to sacrifice. Two male pairs and six female pairs were used.
‡ Standard error.
§ $P < 0{\cdot}01$.
‖ $P < 0{\cdot}001$.
¶ $P < 0{\cdot}05$.

TABLE 4. 5-HYDROXYTRYPTAMINE CONCENTRATIONS OF PERIPHERAL ORGANS AND SPINAL CORD FROM CONTROL AND IMMUNOSYMPATHECTOMIZED RATS AFTER THE ADMINISTRATION OF 10 mg/kg OF JB-516*

Tissue	No. of pairs	5-Hydroxytryptamine (μg/g wet weight $\pm$ standard error)	
		Control	Immunosympathectomy
Submaxillary glands	8	1·89 ± 0·20	2·90 ± 0·51†
Heart	8	0·32 ± 0·03	0·38 ± 0·04‡
Spleen	8	4·39 ± 0·26	2·17 ± 0·48§
Kidneys	8	0·22 ± 0·01	0·25 ± 0·02
Lungs	6	3·68 ± 0·24	4·48 ± 0·76
Liver	6	0·73 ± 0·13	0·89 ± 0·08
Stomach	2	2·12 ± 0·40	2·21 ± 0·09
Small intestine (proximal)	8	3·15 ± 0·42	3·39 ± 0·35
Uterus	4	0·55 ± 0·09	0·80 ± 0·17
Spinal cord	6	0·84 ± 0·14	0·69 ± 0·12

* Catron, β-phenylisopropylhydrazine, i.p., 16–18 hr prior to sacrifice.

† $P < 0·05$, ‡ $P < 0·06$, § $P < 0·01$: The statistical significance was determined by means of the *t*-test in paired experiments.[8]

TABLE 5. 5-HYDROXYTRYPTAMINE CONCENTRATIONS OF PERIPHERAL ORGANS AND SPINAL CORD FROM CONTROL AND IMMUNOSYMPATHECTOMIZED RATS 3hr AFTER THE i.p. ADMINISTRATION OF 150 mg/kg OF 5-HYDROXYTRYPTOPHAN (5-HTP)

Tissue	5-Hydroxytryptamine (μg/g wet weight)					
	Control (2)*		Control + 5-HTP (2)*		Immunosympathectomy + 5-HTP (2)*	
Heart	0·29	0·20	1·10	1·10	1·28	1·43
Submaxillary glands	0·85	1·24	3·04	3·09	3·77	3·35
Lungs	2·09	2·59	3·56	3·38	4·65	4·51
Spleen	3·25	3·03	10·06	8·77	8·12	7·88
Kidneys	0·22	0·27	9·51	9·50	9·44	9·64
Liver	0·54	0·61	1·04	1·73	1·60	1·51
Stomach	1·38	2·00	3·41		3·68	
Small intestine (proximal)	2·51	2·69	3·72	3·90	4·91	5·17
Uterus	0·43	0·39	0·50	0·34	0·49	0·62
Spinal cord	0·53	0·36	0·71	0·48	0·39	0·60

* Number of rats.

no explanation; they merely stated that their observation was in agreement with the findings of Hamberger *et al.*[9] Thompson and Campbell[11] noted elevated intestinal 5-hydroxytryptamine levels in immunosympathectomized mice and presented evidence that this rise seemed to be the result of increased concentrations of 5-hydroxytryptamine per argentaffin cell rather than of a larger cell population.[12, 13] In the study of Hamberger *et al.*[9] no mention was made of an increased fluorescence characteristic of 5-hydroxytryptamine in any other peripheral tissue from immunosympathectomized rats, except for the intestine. In the present study 5-hydroxytryptamine concentrations were not significantly elevated in the segments of the small intestine studied.

However, a significant increase was found in the cecum. In addition, a significant increase was found in the submaxillary glands and the heart.

The administration of β-phenylisopropylhydrazine (JB-516) or 5-hydroxytryptophan to control and immunosympathectomized rats did not elucidate the underlying mechanism responsible for the elevations of the 5-hydroxytryptamine levels of these tissues after immunosympathectomy (Tables 4 and 5). If the increased 5-hydroxytryptamine levels in some of the tissues from immunosympathectomized rats had been due to a derangement of the metabolic pathway of the amine, the administration of these agents could have revealed such a derangement. However, neither the monoamine oxidase inhibitor nor the precursor amino acid accentuated the rise of 5-hydroxytryptamine in tissues from immunosympathectomized rats.

It is difficult to interpret satisfactorily the increased 5-hydroxytryptamine levels in peripheral tissues from immunosympathectomized rats. Innes[14] has shown that in the cat spleen exogenous 5-hydroxytryptamine uses the same receptor as does epinephrine to mediate its effect. He proposed two mechanisms of action for 5-hydroxytryptamine: a direct one on the epinephrine receptor and an indirect one by releasing stored norepinephrine which, in turn, acts on the epinephrine receptor. In a number of other peripheral tissues (rat stomach, dog retractor penis, rabbit aorta, rabbit uterus and guinea pig ileum) epinephrine and 5-hydroxytryptamine act on different receptors.[15] In relatively high concentrations, norepinephrine was shown to be an inhibitor of 5-hydroxytryptophan decarboxylase activity *in vitro*.[16, 17] In the presence of a monoamine oxidase inhibitor and/or 5-hydroxytryptophan, 5-hydroxytryptamine has been found in the perfusate of superior cervical ganglia of cats.[18] It is tempting to make use of one of these divergent and apparently unrelated experimental observations to interpret the findings of the present investigation to propose a connection between the peripheral 5-hydroxytryptamine levels on the one hand, and the norepinephrine concentrations, the sympathetic nervous system or sympathectomy on the other hand.

Before attempting to postulate such a relationship, it is necessary to determine whether the rise of the 5-hydroxytryptamine levels in some peripheral tissues from immunosympathectomized rats is a direct result of the NGF-antiserum administration or whether it is due to the fact that these tissues are largely devoid of sympathetic innervation. The significant increases of 5-hydroxytryptamine occurred in those tissues (submaxillary glands and heart) which are nearly completely sympathectomized by the NGF-antiserum administration.[1, 19] This observation presented a rationale to explore the effect of surgical sympathetic denervation on the 5-hydroxytryptamine levels of submaxillary glands of rats. The results of this investigation are published in the following paper.[20]

Acknowledgements—The author wishes to thank Mrs. Anna Poliszczuk and Mrs. Alice H. Jones for technical assistance.

REFERENCES

1. G. I. KLINGMAN, *J. Pharmac. exp. Ther.* **148**, 14 (1965).
2. G. I. KLINGMAN, *Pharmacologist* 7 (2), 157 (1965).
3. G. I. KLINGMAN, *Biochem. Pharmac.* **15**, 1729 (1966).
4. G. I. KLINGMAN and J. D. KLINGMAN, *Life Sci.* **4**, 2171 (1965).
5. G. I. KLINGMAN, *First Int. Meet. of the Int. Soc. Neurochemistry. Abstr.* p. 116 (1967).

6. G. I. KLINGMAN and J. D. KLINGMAN, *J. Neurochem.*, **16**, 261 (1969).
7. D. F. BOGDANSKI, A. PLETCHER, B. B. BRODIE and S. UDENFRIEND, *J. Pharmac. exp. Ther.* **117**, 82 (1956).
8. H. C. BATSON, *An Introduction to Statistics in the Medical Sciences*, 5th edn. Burgess Publishing Co., Minneapolis, Minn. (1961).
9. B. HAMBERGER, R. LEVI-MONTALCINI, K-A. NORBERG and F. SJÖQVIST, *Int. J. Neuropharmac.* **4**, 91 (1965).
10. L. L. IVERSEN, J. GLOWINSKI and J. AXELROD, *J. Pharmac. exp. Ther.* **151**, 273 (1966).
11. J. H. THOMPSON and L. B. CAMPBELL, *J. Pharm. Pharmac.* **18**, 753 (1966).
12. J. H. THOMPSON, *Archs Path.* **83**, 415 (1967).
13. J. H. THOMPSON and L. B. CAMPBELL, *Biochem. Pharmac.* **17**, 175 (1968).
14. I. R. INNES, *Br. J. Pharmac. Chemother.* **19**, 427 (1962).
15. I. R. INNES, *Br. J. Pharmac. Chemother.* **21**, 427 (1963).
16. A. YUWILER, E. GELLER and S. EIDUSON, *Archs Biochem. Biophys.* **80**, 162 (1959).
17. J. A. BUZARD and P. D. NYTCH, *J. biol. Chem.* **234**, 884 (1959).
18. S. B. GERTNER, M. K. PAASONEN and N. J. GIARMAN, *J. Pharmac. exp. Ther.* **127**, 268 (1959).
19. R. LEVI-MONTALCINI and P. U. ANGELETTI, *Int. J. Neuropharmac.* **1**, 161 (1962).
20. G. I. KLINGMAN and J. D. KLINGMAN, *Biochem. Pharmac.* **18**, 2069 (1969).