NOTE

PITUITARY ADRENOCORTICOTROPHIC HORMONE LIBERATION BY SEROTONIN *

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Accumulating evidences show that serotonin, an important neuro-endocrine factor in lower animals, is also involved in the regulation of endocrine system in higher vertebrates. Stimulation by serotonin of release of epinephrine from adrenal medulla has been observed in cats (Reid, 1952), rats (Kobayashi *et al.*, 1960; Ui *et al.*, 1960), and dogs (Columbo *et al.*, 1960). Quite recently, an increment of blood insulin-like activity in serotonin-treated rats, suggesting increased secretion of insulin from pancreatic islet, was demonstrated in this laboratory (Ui and Kobayashi, 1961). On the other hand, release of a hyperglycemic principle from pancreatico-duodenal area following an injection of pituitary growth hormone has been discussed in respect to serotonin (Sirek, 1957), and increased excretion of 5-hydroxyindoleacetic acid in rabbit urine after injection of the growth hormone has been demonstrated (Laborit *et al.*, 1959).

The present paper describes the release of pituitary adrenocorticotrophic hormone by serotonin, in confirmation of the result reported by Moussatché and Pereira (1957).

EXPERIMENTAL

Throughout the experiment adult male rats of Wistar strain, carefully bred in this laboratory and maintained on a constant stock diet (Oriental Yeast & Co.) with free access to clean drinking water, were used. They were kept in a constant temperature quarter of $27 \pm 1^\circ\text{C}$ for at least 10 days prior to, and during the conduct of the experiment.

Hypophysectomy was carried out through parapharyngeal approach (Imamichi, 1955) under pentobarbital anesthesia. Twenty-three hrs. after hypophysectomy, these rats or non-operated controls which had been fasted for more than 24 hrs. were anesthetized with intraperitoneal sodium pentobarbital (45 mg per kg of body weight). The left adrenal was removed from each rat 40 mins. after the anesthesia, and then 1 mg of serotonin creatinine sulfate (435 μg as free base; a commercial preparation of Nutritional Biochemicals Corporation) or its equivalent was injected subcutaneously in volume of 0.2 ml. Control rats received physiological saline in place of serotonin solution. Exactly 1 hr. later each rat was decapitated, and the right adrenal was removed.

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The ascorbic acid content of adrenal gland was determined by the 2, 4-dinitrophenylhydrazine method of Roe and Oesterling (1944), and the response was expressed as the difference in content of ascorbic acid between the left and the right adrenal of the same rat.

In accord with the results of others, preliminary experiment revealed that the adrenal ascorbic acid content of individual rat was subject to large variation. Therefore, a further refinement by the usage of litter-mates was tried in the present experiment; *i.e.*, in the experiment II, 5 litters of 4 were allotted evenly to the 4 experimental groups.

Completeness of hypophysectomy was confirmed by means of post mortem examination of operated rats.

RESULTS AND DISCUSSION

Table 1 shows the effect of 1 mg of subcutaneous serotonin on adrenal ascorbic acid content of intact male rats. These data were derived from the experiment in which several litter-mates of unequal size were allotted to two experimental groups without taking into consideration of any balance within litters

Table 1. Effect of serotonin on adrenal ascorbic acid content of intact rats (Experiment I)

Group	Control saline injected	Serotonin 1 mg per rat, s.c.
Change in adrenal ascorbic acid*	-80.64 ± 19.78 (10)**	-133.56 ± 16.43 (11)
	$P < 0.10$	

* concentration in right adrenal — concentration in left adrenal; in μg ascorbic acid per 100 mg wet tissue

** mean $\pm$ standard error of the mean; figures in parentheses denote number of animals

(Experiment I). The difference of ascorbic acid content between the left and the right adrenal gland is greater in serotonin-treated than in control saline injected group. Whereas condition of homoscedasticity is fulfilled between the two group values, test of significance of the difference between group means reveals that they are significant only at the level of 10%. Larger variation within groups is apparently responsible for the reduction of efficiency in the assay, and the necessity of further refinement in the conduct of assay is suggested when smaller number of animals is to be used. Throughout the experiment no consistent difference in mean adrenal weight was noticed between serotonin-treated and control groups.

Although role of adrenal ascorbic acid in the trophic action of pituitary adrenocorticotrophic hormone remains to be elucidated, allowance will be made to postulate that enhanced reduction in adrenal ascorbic acid content of serotonin-treated rats may result from an increased secretion of adrenocorticotrophic hormone from anterior pituitary, and/or direct stimulation, through the same biosynthetic mechanism, of corticosteroid production by serotonin itself.

In an attempt to identify the mode of action of serotonin in adrenal response, a litter-mate controlled experiment was conducted in which serotonin was injected into hypophysectomized rats (Group HS) and their litter-mates with intact hypophysis (Group NS); the results being shown in Table 2. Hypophy-

Table 2. Effect of serotonin on adrenal ascorbic acid content of intact and hypophysectomized rats (Experiment II)

Group		NN intact 0	NS intact 1 mg, s.c.	HN ectomized 0	HS ectomized 1 mg, s.c.	sum
Litter	1	— 42.0*	— 104.7	49.1	69.6	— 28.0
	2	— 125.7	— 246.3	30.2	— 15.6	— 357.4
	3	— 51.0	— 150.3	17.5	67.6	— 116.2
	4	— 75.2	— 70.1	48.5	— 0.3	— 97.1
	5	— 90.8	— 184.5	43.5	13.9	— 217.9
sum		— 384.7	— 755.9	188.8	135.2	—

* concentration in right adrenal — concentration in left adrenal; in μg ascorbic acid per 100 mg wet tissue

Table 3. Analysis of variance of the data in Table 2

Nature of variation	Degree of freedom	Variance ratio	Level of significance
Serotonin	1	7.78	$P < 0.05$
Hypophysectomy	1	92.53	< 0.001
Serotonin $\times$ Hypophysectomy	1	4.35	< 0.10
Litter	4	3.53	< 0.05
Error	12	—	—
Total	19		

sectomized (Group HN) and intact (Group NN) litter-mates served as their respective controls. Analysis of variance in Table 3 reveals that effect of hypophysectomy is highly significant ($P < 0.001$), while the effect of serotonin and that of litters are significant at the level of 5% (in the present experiment, effect of litters and that of experimental days are confounded). Furthermore, the interaction between "serotonin" and "hypophysectomy" is found to be significant, even though at the level of 10%. Further analysis reveals that the differences between Groups NS and HS, NN and HS, NN and HN are all significant at the level of 0.1%, while the difference between Groups NS and NN is significant at 1% level. On the contrary, the difference between Groups HS and HN is far from significant. It is thus evident that litter-mate control of the experiment fairly improved the efficiency of the assay. In view of the absence of serotonin effect in hypophysectomized animals, it should safely be deduced that serotonin does induce liberation of adrenocorticotrophic hormone from pituitary in the intact rats.

The results mentioned above confirm that of Moussatché and Pereira (1957), which showed the necessity of intact hypophysis in the demonstration of serotonin-induced adrenal ascorbic acid depletion, and is also in accord with the result of Smelik and de Wied (1958), in which serotonin picrate was shown to be incapable of releasing adrenocorticotrophic hormone in rats treated with

prednisolone or in hypophyseo-hypothalamic connection-severed rats. With the objection in mind that serotonin might exert its corticosteroidogenetic effect via a different route from that of adrenocorticotrophic hormones, the present result may be interpreted not to support the concept that serotonin itself exerts direct corticosteroidogenetic effect on adrenals, as has been proposed by Rosenkrantz (1959) in his *in vitro* experiment. Although the increments in adrenal ascorbic acid of the hypophysectomized rats tended to be smaller in the serotonin-treated group than in the controls, the large within-group variation nullified the significance of the difference. Furthermore, larger variance due to interaction between "serotonin" and "hypophysectomy" disapproves of, although not conclusively, the direct stimulative action of serotonin on adrenal cortex under the condition of the present experiment.

Recently several reports showing the effects of adrenal hormones on body serotonin levels and on serotonin forming enzyme systems have been published (Hicks and West, 1958; Yuwiler *et al.*, 1959; Schmid *et al.*, 1960; Enerbäck, 1960). These papers may lend some supports to the involvement of serotonin in the regulatory mechanism of corticosteroid production. It is tempting to speculate that serotonin might contribute to the production of corticosteroids through accumulation of activators such as cyclic adenylylate or reduced triphosphopyridine nucleotide in adrenal tissues, although species- and organ-specificities in the serotonin action should never be overlooked (Sweat and Lipscomb, 1955; Haynes *et al.*, 1959; Mansour *et al.*, 1960; Columbo *et al.*, 1960). Although the present result suggests the contrary, this possibility should be sought elsewhere, and the participation of non-specific systemic stress in the adrenal ascorbic acid depletion should be noticed in the interpretation of this sort of experimental results.

SUMMARY

Results were obtained to support the view that the presence of intact pituitary gland must be a prerequisite to the adrenal ascorbic acid depletion after administration of serotonin into rats. Possibility of the direct stimulative action of serotonin on adrenal cortex was not supported, though not disapproved of, under the condition of the present experiment.

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