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Failure of Acute Bilateral Adrenalectomy to Influence Brain Serotonin Levels in the Rat. (25648)

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Recently, De Maio reported differential increases of serotonin concentration in pooled fractions of the base, hemispheres, and medulla oblongata of the rat brain 24 hours after bilateral adrenalectomy or hypophysectomy (1). His report suggests a possible influence of endocrine function on brain serotonin metabolism. It was considered of interest to repeat the studies with the acute, bilaterally adrenalectomized rat using a chemical, fluorimetric procedure instead of a bioassay method. Since the surgical procedures employed by De Maio are well known to activate the pituitary-adrenal cortex system, it was considered important to include a sham operated group of animals in order to distinguish specific from nonspecific effects of ablative surgery. In addition our estimations have been made on the individually treated animals, and the results subjected to standard statistical analysis.

Methods. Two studies were made on the effect of bilateral adrenalectomy on brain serotonin concentration. In the first experiment 15 albino rats (Sprague-Dawley strain), weighing between 150 and 200 g, were divided into 3 groups: control, sham operated, and bilateral adrenalectomized. Ether was used as anesthesia in all of the operations. The animals were sacrificed by decapitation 24 hours after surgery. The brain was immediately removed, weighed, and homogenized in 3 volumes of 0.1 N HCl. Serotonin was assayed according to the indirect method of Weissbach, Waalkes, and Udenfriend, involving extraction with butanol(2).

Results. The control group exhibited a mean concentration of 0.65 μg of serotonin per gram of fresh brain tissue. Mean values found in the sham operated and adrenalectomized groups were 0.61 and 0.67 μg of serotonin per gram of fresh brain tissue, respectively. Values for groups of animals were not significantly different from each other.

The above experiment was repeated with addition of a fourth group injected with 100 mg of iproniazid/kilo of body weight 18 hours prior to sacrifice. As in the first experiment, there was no significant difference in brain serotonin concentrations of control, sham operated, and bilateral adrenalectomized groups. The iproniazid treated group showed a 165% increase in brain serotonin over that of control group ($P = 0.01$). Table I summarizes means, standard deviations, and ranges of the groups tested. The data were analyzed by use of the Mann Whitney U-test(3). P values in Table I refer to comparisons of experimental groups to control group. We have also demonstrated that reserpine treated animals have the expected depletion of brain serotonin using the fluorimetric method(4).

To make direct comparison of our data with that of De Maio's, 10 rat brains were separated and pooled into the 4 fractions described by De Maio. Mean weights for these frac-

TABLE I. Serotonin Levels, in μg/g Fresh Tissue, of Various Groups under Investigation.

	Control	Sham-operated	Bilateral adrenalectomy	Iproniazid
Mean	.65	.61	.67	
Range	.60–.74	.58–.64	.62–.72	
Stand. dev.	±.047	±.024	±.036	
No. of animals used	5	5	5	
P		>.20	>.20	
Mean	.43	.50	.52	1.14
Range	.37–.51	.35–.63	.40–.66	.99–1.38
Stand. dev.	±.057	±.101	±.092	±.129
No. of animals used	5	5	5	5
P		>.20	>.10	.01

tions and their standard deviations were: 0.41 ± 0.09; 0.26 ± 0.04; 0.32 ± 0.02; and 1.08 ± 0.07 g of tissue for the base, medulla oblongata, cerebellum, and hemispheres fractions, respectively. Recalculating De Maio's data using the mean weights for our pooled fractions, we obtained 0.69, 1.43, and 0.87 μg serotonin per gram of tissue for De Maio's control, bilateral adrenalectomized, and hypophysectomized groups, respectively.

Clearly our results are inconsistent with De Maio's findings. Neither the sham operated nor the bilateral adrenalectomized groups showed a significant increase in brain serotonin level over the untreated group. As expected the iproniazid treated group (which was carried as a control for the reliability of the fluorimetric method) gave the expected significant increase in brain serotonin level (5).

In preparation of brain extracts De Maio used 80% acetone twice for 24 hours. According to Amin, *et al.*(6), use of 80% acetone instead of 95% acetone for preparation of brain extracts greatly enhances the extraction of substance P. Qualitatively the estrus rat uterus responds to substance P in a manner similar to serotonin, but these substances may be distinguished by use of serotonin antagonists(6). It is possible that the apparent increases of serotonin observed by De Maio using bioassay methods were due to substantial increases in substance P and not in serotonin. It is unlikely that closely related derivatives of serotonin could account for the differences observed. Bases which contain the 5-hydroxyindole group would not give a clear response in the bioassay(7), but would contribute towards estimation of serotonin by fluorimetric procedures.

Summary. These experiments find no effect on brain serotonin level as a result of acute bilateral adrenalectomy when the chemical fluorimetric procedure is used for estimating serotonin.

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