ADRENAL EPINEPHRINE AND NOREPINEPHRINE CONTENT FOLLOWING DENERVATION AND BARBITURATES

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TISSUE CONCENTRATIONS of norepinephrine in heart, striated muscle, kidney, and spleen [11, 23, 24] are known to depend on an intact postganglionic autonomic innervation. This is not true for the preganglionic sympathetic fibers innervating these organs, and division of the preganglionic neurons to these same tissues is not followed by a sustained change in their norepinephrine content [11, 23, 24].

Because the innervation of the adrenal medulla is by preganglionic fibers, and because of the occurrence in the adrenal medulla of chromaffin cells rather than conventional postganglionic neurons, it was of interest to determine if division of the preganglionic innervation of the adrenal gland resulted in any change in adrenal catecholamines. The unique efficiency of the adrenal medulla in completing the biosynthesis of epinephrine from norepinephrine [1, 12, 17] made an examination of the adrenal concentration of epinephrine under these circumstances of particular interest.

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Another important consideration in the experimental approaches to adrenal medullary physiology is the widely held conviction that barbiturate anesthesia will acutely stimulate the massive release of catecholamines and that immediate suppression of adrenal catecholamine concentrations results. This was first suggested by Lund in 1951 [20] on the basis of adrenal catecholamine levels of dogs killed by shooting. Because we cannot reconcile Lund's findings with reports of barbiturate suppression of adrenal catecholamine secretion rates [16, 26], we have examined this point in conscious dogs killed by rapid exsanguination.

METHODS

Two groups of 10 healthy adult mongrel dogs, weighing from 10 to 15 kg., and a third group of 5 dogs were used. In the first group the animals were given a large dose of barbiturate intravenously; the adrenals were immediately harvested, weighed, and homogenized; and two extractions with 50 ml. of 10% trichloracetic acid were immediately carried out. The extracts were neutralized; the catecholamines were adsorbed onto alumina, and after elution with two 5-ml. portions of 0.2 N acetic acid were analyzed

fluorometrically for epinephrine and norepinephrine following oxidation to their trihydroxyindole forms by the method of Lund [19], as modified by Crout [5] and von Euler and Lishajko [10]. A Farrand photofluorometer was used to measure fluorescence of the samples, and two filter systems of fixed wavelengths were used for the differential estimation of epinephrine and norepinephrine. Recovery of epinephrine and norepinephrine added to the acid homogenate varied between 83% and 100%. No correction was made for the incomplete recovery. We have previously used this technique for catecholamine determinations of monkey tissues and found it to be suitably sensitive and reproducible [13].

In the second group the dogs were anesthetized with 20 mg. per kilogram of intravenous pentobarbital and the right adrenal gland was surgically removed through a transverse upper abdominal incision. The innervation of the left adrenal gland was then exposed and meticulously divided at a point midway between the diaphragm and the left adrenal gland in the manner described by Vogt [25]. Reflex excitation of the adrenal release of epinephrine and norepinephrine is completely destroyed by this technique and on this basis we feel confident that virtually complete adrenal denervation has been achieved in this study [14]. The abdomen was closed and the dog allowed to recover. Three weeks later the denervated left adrenal was removed and the dog sacrificed by the same technique used in the control animals. Three weeks was the period employed by Vogt [25] in her studies on the denervated adrenal gland, and it is thought that this is ample time for recovery of the adrenal medulla from surgery; moreover, significant regeneration of the divided splanchnic nerve would not be expected at three weeks.

To determine any influences of the barbiturate anesthesia used in the previous experiments a series of trained dogs were placed on a table and the right femoral artery exposed after 1% local lidocaine (Xylocaine) infiltration anesthesia. A large Teflon catheter was passed into the artery and the animals bled rapidly and maximally into a flask at less than 1 atm. of pressure. The bleeding times were 5 minutes or less. The adrenal glands were immediately harvested and analyzed for epinephrine and norepinephrine in a manner identical to that described above.

RESULTS

The mean weights of the adrenal glands studied were similar in all groups. As seen in Table 1, the mean concentrations of epinephrine and norepinephrine from the right and left adrenal glands of the control group of animals are comparable. The mean values from the chronically denervated glands and the corresponding controls are shown in Table 1. The epinephrine and norepinephrine content of these two groups is comparable to the control values. The mean epinephrine and norepinephrine contents seen in the surgically removed adrenals (Table 1) do not vary significantly from the controls.

In the ten adrenal glands removed after bleeding without barbiturate anesthesia both epinephrine and norepinephrine levels are comparable to those seen in the other groups.

Table 1. Mean Catecholamine Content of Right and Left Adrenal Glands in 10 Control Animals, 10 Surgically Excised Glands, 10 Chronically Denervated Glands, and 10 Glands After Bleeding (μg./gm. wet tissue, ± 1 S.D.)

	Right Control	Left Control	Surgically Removed	Chronically Denervated	Exsanguination Local Anesthesia
Norepinephrine	161 ± 65	166 ± 68	146 ± 58	147 ± 51	174 ± 33
Epinephrine	752 ± 173	819 ± 209	649 ± 204	864 ± 236	715 ± 234

Group comparisons between each control group and the other three groups reveal no significant differences.

DISCUSSION

Up to this time there has been no separate measurement of the levels of both epinephrine and norepinephrine in the denervated adrenal gland. This investigation has been conducted to furnish such data in order to interpret accurately the significance of decreased secretory rates of catecholamines from the denervated adrenal gland. We believe these data will be of eventual importance in helping to establish whether or not so-called "direct" influences, acting independently from the nervous system, are important in the physiological secretion of the adrenal medulla. Although one might deduce from observations of Mirkin [21] and Callingham and Mann [3], and indirectly from those of Cammanni et al. [4], that the denervated adrenal gland's content of epinephrine and norepinephrine is unchanged, separate measurements of the adrenal levels of both of these catecholamines after denervation have not been recorded. It is well known from the observations of Bennett [2] and Lever [18] that terminal preganglionic dendritic arborizations are intimately applied to the individual chromaffin cells of the adrenal medulla. With this in mind it has seemed of particular interest to determine if loss of neural activity in these fibers will result in a decrease in the concentration of epinephrine and norepinephrine in the chromaffin cells. It is probable that the rate of biosynthesis of catecholamines by the adrenal medulla is increased by stimulation of the adrenal innervation, although evidence used to support this contention is somewhat crude [15]. Whether or not prolonged absence of nervous stimuli to the adrenal gland will result in a decreased adrenal catecholamine level does not appear to have been examined rigorously.

We feel that our observations demonstrate that the denervated adrenal gland will maintain physiological tissue levels of epinephrine and norepinephrine. This might also be inferred from old observations of Vogt's [25], in which chronically denervated cat adrenal glands were able to release large quantities of catecholamines in response to the glandular stimulus of potassium salts. Any decrease in the adrenal medullary secretion of epinephrine and norepinephrine by the denervated adrenal gland such as that shown by Vogt [25] and Duner [6] cannot, therefore, be attributed to decreased tissue levels of these catecholamines. Restoration of epinephrine levels in the adrenal gland following reserpine administration is not retarded in the denervated adrenal gland [3]. It is, therefore, probable that the biosynthesis of epinephrine by the chromaffin cells occurs after preganglionic nerve section. Our results are in agreement with this idea and with unpublished observations alluded to by von Euler [9].

Of importance to the interpretation of adrenal catecholamine levels in glands obtained under anesthesia is the question of what influence barbiturates or other general anesthetic agents might have on adrenal catecholamine content. Old observations of Elliott [7] indicate that ether anesthesia will deplete the innervated adrenal medulla of epinephrine. More recently Elmes and Jefferson [8] have shown that pentobarbital has only a slight effect on adrenal catecholamines, and Price et al. [22] failed to demonstrate any secretory burst of catecholamines into the arterial plasma in human subjects given barbiturate anesthesia. In spite of these observations, well-known findings of Lund have been cited frequently as evidence that barbiturates acutely stimulate massive release of epinephrine and norepinephrine and have led to repeated criticism of tissue catecholamine levels obtained under anesthesia [20]. We are unable to confirm Lund's results in conscious dogs in the animals of this study which were sacrificed by sudden exsanguination. The adrenal secretory rates of catecholamines in response to hemorrhage in conscious animals are not great enough [16, 27] to account for a depression of the magnitude suggested by Lund [20], and, furthermore, study of dogs under barbiturate anesthesia has demonstrated only suppression of catecholamine secretion in response to barbiturates [16, 26]. The results reported in this study indicate that there is no suppression of adrenal epinephrine and norepinephrine con-

tent in response to light barbiturate anesthesia and that levels obtained under these conditions accurately represent catecholamine levels of the conscious animal. In all other respects the levels of adrenal catecholamines which we report in this study are comparable to those of the dogs studied by Lund [20].

SUMMARY

The concentration of epinephrine and norepinephrine in the adrenal gland of the dog is comparable in the left and right glands. Denervation of the left adrenal gland does not decrease the levels of these adrenal catecholamines three weeks after denervation. These findings are in keeping with the concept that catecholamine biosynthesis by the adrenal gland is not dependent on adrenal innervation. Other evidence supporting this concept is reviewed.

In contrast to prevailing opinion we have found that the catecholamine content of adrenal glands obtained from conscious dogs is comparable to that in animals with general barbiturate anesthesia.

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