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THE EFFECT OF ADRENALINE, *NOR*ADRENALINE, AND *ISO*PROPYL *NOR*ADRENALINE ON THE ASCORBIC ACID CONTENT OF THE RAT'S ADRENAL

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Many of the observations that have followed the original work of Szent-Györgi on the ascorbic acid content of the adrenal gland have been directed towards a study of the significance of this vitamin in relation to the physiological state of the adrenal cortex. A wide variety of changes in environmental conditions are known to produce a depletion of the ascorbic acid content of the adrenal gland. The fall in the concentration of ascorbic acid in the adrenal has been used as a measure of enhanced adrenal cortical activity, and this enhancement has been presumed to be caused by the release of adrenocorticotrophic hormone from the anterior pituitary (Sayers, Sayers, Liang, and Long, 1945).

The injection of adrenaline has been shown by Vogt (1944) to produce a large increase in the amount of cortical hormone in the adrenal effluent of the dog, and Long and Fry (1945) observed a fall in the concentration of ascorbic acid in the adrenals of rats after the subcutaneous injection of this amine. Nasmyth (1949), in a study of the effect of some sympathomimetic amines, also reported a large depletion in the level of ascorbic acid in the adrenal of the rat after the subcutaneous injection of adrenaline, and a smaller depletion after that of *nor*adrenaline

In view of the increasing interest in substances which will release adrenocorticotrophic hormone (ACTH) when given in non-toxic doses it was decided to extend these studies, and particularly to compare the release of ACTH induced by *iso*propyl-*nor*adrenaline with that seen after adrenaline and *nor*adrenaline.

METHODS

Female rats of the Wistar strain (body weight 140–200 g.) were used for these investigations. The day before the experiment the animals were removed from the stock cages, weighed, marked, and then placed in a room thermostatically controlled at about 22° C. They were left undisturbed overnight and used with a minimum of handling on the day of the experiment.

The drugs were administered by subcutaneous injection, and after the required time interval had elapsed (1–4 hours) each animal was killed by a sharp blow on the head and then decapitated. The left adrenal gland was rapidly removed, carefully stripped of all extraneous tissue, and weighed to the nearest 0.1 mg. The gland was immediately immersed in a small volume of 4.5 per cent (w/v) trichloroacetic acid, homogenized, and the volume made up to 10 ml. with 4.5 per cent trichloroacetic acid. The ascorbic acid was estimated by the method of Roe and Kuether (1943), and the content of the gland was expressed as mg. ascorbic acid per 100 g. tissue.

Eighteen rats were demedullated bilaterally by enucleating the gland from the capsule and then allowing sufficient time to elapse for regeneration of cortical tissue. These animals were kept in the warm room for three weeks after the operation and thereafter subjected to normal room temperatures. During the first week they were given 0.9 per cent saline to drink. They were used about forty-five days after operation; good regeneration had taken place by this time.

The amines used were *l*-adrenaline, *l-nor*adrenaline *d*-bitartrate monohydrate, and *dl-iso*propyl-*nor*adrenaline hydrochloride (isoprenaline). A stock solution of each amine in dilute hydrochloric acid was made containing 0.1 g. of the base per 100 ml. and the *p*H adjusted to 3–4. Immediately before injection appropriate dilutions in physiological saline were made (usually 1/10 or 1/20) and the *p*H was adjusted to about 7 with sodium bicarbonate. The doses varied between 0.005 and 0.060 mg. base per 100 g. body weight.

In order to assess the extent to which the experimental procedure might have produced emotional excitement, with a consequent effect on the ascorbic acid content of the adrenals, a group of rats was injected with 0.9 per cent saline and killed at various times thereafter.

TABLE I

Ascorbic acid concentration (mg./100 g. fresh gland) in the adrenal gland after subcutaneous injection of saline, *l*-adrenaline, *l-nor*adrenaline, and isoprenaline into normal rats and into rats whose adrenals were demedullated. Control rats had an ascorbic acid content of 420±24 mg./100 g.

Drug	Dose mg./100 g.	Ascorbic acid (mean and S.E. of mean) at various times after injection. Number of rats in parentheses		
		1 hr.	2 hr.	3 hr.
0.9% saline	0.9	383±16(13)	359±9(10) 396±24(7)*	375±28(6)
l-adrenaline	0.01	283±7(6)	258±17(9) 279±17(6)*	300±16(10)
	0 02	262±11(5)	230±16(4)	250±8(5)
*l-nor*adrenaline ..	0.0075		354±14(8)	
	0.015	319±17(6)	283±6(6)	340±21(4)
	0.030	290±20(3)	283±6(9)	275±8(5)
	0 060		278±13(8)	287+8(4)
isoprenaline	0.005	269±12(13)	300±9(10)	292±16(7)
	0.010	267±17(6)	243±11(13)	300±11(6)
	0.015	271±12(11)	257±14(10) 260±17(5)*	288±15(7)
	0.020	249±14(6)	213±16(6)	296±12(6)
	0.030	234±4(3)	224±15(6)	234±16(5)

* Rats whose adrenals had been demedullated 45 days previously.

RESULTS

A series of animals which received no injection but were otherwise kept under the same environmental conditions as the injected groups had an average concentration of ascorbic acid in the adrenal gland of 420 mg./100 g. (S.E. of mean ±24). It will be seen from Table I that the injection of saline provided a sufficient stimulus to cause some depletion of the ascorbic acid. The minimum figure determined was at the two-hour period. This reduction in the level of the ascorbic acid was small,

however, when compared with that produced by the various amines (see Fig. 1). After four hours the values had usually returned to the pre-injection level.

l-Adrenaline in doses of 10 μg. and 20 μg. per 100 g. led to a large depletion. After *l-nor*adrenaline the depletion was less pronounced, and a dose as high as

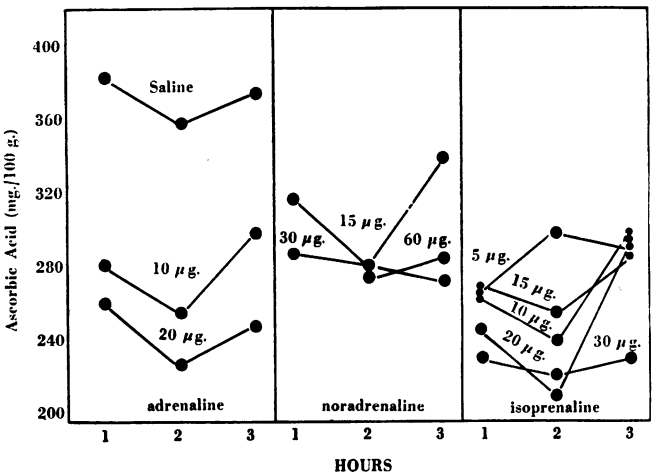
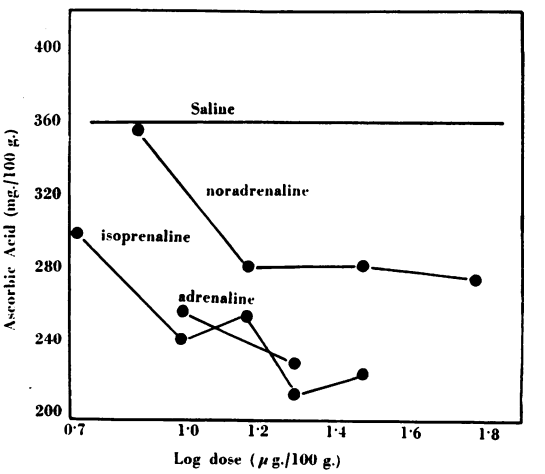


FIG. 1.—Concentrations of ascorbic acid (mg./100 g.) in rats' adrenal glands after subcutaneous injection of saline, adrenaline, *nor*adrenaline, and isoprenaline plotted against time in hours.

60 μg. per 100 g. failed to produce a response equivalent to that obtained with much smaller amounts of adrenaline. Isoprenaline, however, in spite of being injected as a racemic mixture of which the *d*-isomer is probably inactive, produced effects as large as did equal doses of *l*-adrenaline.

FIG. 2.—Adrenal ascorbic acid (mg./100 g. tissue) two hours after injection of saline, adrenaline, *nor*adrenaline, and isoprenaline plotted against log dosage.



The effect was fully developed two hours after the injection, except that the smallest dose of isoprenaline had its maximum effect earlier. The values determined two hours after injection were used for plotting a dose-response curve for each drug.

These dose-response curves (Fig. 2) reveal large differences in potency between *l-nor*adrenaline on the one hand and *l*-adrenaline and isoprenaline on the other. Comparable effects were obtained with 10 μg. *l*-adrenaline and 10 or 15 μg. isoprenaline, and also with 20 μg. *l*-adrenaline and 20 or 30 μg. isoprenaline. As isoprenaline was used in the racemic form, it would appear that it is at least as active as adrenaline in producing depletion of the ascorbic acid in the rat's adrenal. *Nor*adrenaline was less effective, and its efficacy did not increase significantly with the larger doses (up to 60 μg./100 g.).

In order to determine whether the high activity of isoprenaline was a property of the amine itself or due to the liberation of endogenous adrenaline, experiments were carried out on demedullated rats. The results obtained are marked with an asterisk in Table I. Two hours after a saline injection the ascorbic acid level in the demedullated group was slightly, though not significantly, higher than in the normal unoperated group. After injection of adrenaline and isoprenaline, however, the fall in ascorbic acid was virtually the same in demedullated and in normal rats.

Discussion

Equal doses of *l*-adrenaline and *dl-iso*propyl-*nor*adrenaline (isoprenaline) produced comparable falls in the ascorbic acid concentration of rats' adrenals. On the assumption that the *dextro*-isomer of isoprenaline is pharmacologically inactive, it would appear that the *laevo*-compound is more potent than *l*-adrenaline in depleting adrenal ascorbic acid, or, if we accept the usual interpretation of this depletion, in releasing ACTH. In confirmation of Nasmyth's work (1949), *l-nor*adrenaline was found much less potent than *l*-adrenaline. The fact that a fourfold increase in the dose of *nor*adrenaline was not associated with an appreciable increase in the loss of ascorbic acid may be connected with the observation that *l-nor*adrenaline, in spite of its greater pressor effect, is less toxic than *l*-adrenaline (Tainter, Tullar, and Luduena, 1948). In the anaesthetized dog, Vogt (unpublished) was unable to obtain proof of enhanced adrenocortical secretion after infusion of doses of *l-nor*adrenaline which were much larger than the amounts of adrenaline required to accelerate cortical secretion. The lack of correlation between pressor effect and action on the hypophysial-adrenal system is even more striking when *nor*adrenaline is being compared with isoprenaline, since the latter drug lowers the blood pressure. It is because of this depressor effect that the experiments on isoprenaline were repeated in rats with demedullated adrenals, as it was conceivable that the fall in blood pressure which follows injection of isoprenaline might give rise to a compensatory secretion of adrenaline with consequent release of adrenocorticotrophic hormone. However, as the same fall in adrenal ascorbic acid was obtained after isoprenaline in normal and in demedullated rats, it would appear that any effect due to release of endogenous adrenaline was insignificant compared with the direct effect of the isoprenaline.

Ahlquist (1948), in a study of receptors for sympathomimetic amines, attempted to classify the effects of amines of this type on different physiological functions and found some tissue responses highly sensitive to *nor*adrenaline and little sensitive to isoprenaline, whereas the reverse was true for other responses. He postulated two types of cell receptors to account for these differences, calling the first type α and the

second β receptors. According to this classification it would be possible to consider the hypothetical receptors responsible for release of adrenocorticotrophic hormone as belonging to the β class.

From the results of the saline injections it is apparent that even under conditions of minimum handling there was some depletion of adrenal ascorbic acid; this stresses the necessity of careful control of all manipulations in experiments which measure the release of ACTH in conscious animals.

Summary

1. The concentration of ascorbic acid in the adrenal gland of the rat was determined after the subcutaneous injection of *l*-adrenaline, *l-nor*adrenaline, and *dl-iso*propyl-*nor*adrenaline (isoprenaline).

2. A large depletion was observed two hours after the injection of adrenaline and a much smaller depletion after that of *nor*adrenaline.

3. Isoprenaline is as potent as *l*-adrenaline in depleting ascorbic acid in the adrenal gland, in spite of the fact that it consists of two isomers, one of which may be inactive.

4. The depletion of ascorbic acid after the injection of adrenaline and isoprenaline is not diminished by adrenal demedullation. The effect of the injection of isoprenaline cannot therefore be explained by the release of endogenous adrenaline.

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