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THE CONCENTRATION OF ADRENALINE IN THE PLASMA OF RABBITS TREATED WITH RESERPINE

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Doses of 1 to 2.3 mg./kg. of reserpine given intravenously to rabbits raised the plasma adrenaline. The noradrenaline concentrations were too near the threshold of the method to decide whether any alterations followed the injection of reserpine. Control experiments, in which blood samples were taken from rabbits but no drug was given, showed only a very slight rise in adrenaline concentration after the first bleeding.

It is known that reserpine causes a loss of adrenaline and noradrenaline from the adrenal medulla (Holzbauer and Vogt, 1956 ; Carlsson and Hillarp, 1956), from the brain (Holzbauer and Vogt, 1956 ; Shore, Olin, and Brodie, 1957), from the heart (Bertler, Carlsson, and Rosengren, 1956 ; Paasonen and Krayer, 1957), and from peripheral sympathetic tissue (Muscholl and Vogt, 1957a and b). The animal which has received reserpine, however, does not exhibit very obvious signs of increased sympathetic activity such as would be expected during a discharge of adrenal medullary amines. In fact, the well-known signs of miosis and relaxation of the nictitating membrane produced by injections of reserpine have usually been interpreted as signs of diminished sympathetic activity. The question might therefore be asked whether the amines lost from the tissues are really released into the circulation or whether the losses are caused by inhibition of amine synthesis. There are several observations which favour the view that there is genuine release of amines after an injection of reserpine. Everett, Toman, and Smith (1957) have shown that, 30 min. after a very large dose of reserpine, mice pass through a phase of piloerection which is followed by a long period during which the piloerector reflex to cold is abolished. Kuschke and Frantz (1955) demonstrated a hyperglycaemic effect of reserpine in the rabbit which was sensitive to ergot alkaloids ; the effect was not abolished by splanchnotomy. Rises in blood pressure in the rat and the spinal dog, and contractions of the denervated nictitating membrane of the cat, have been reported to follow injections of reserpine (de Jongh and v. Proosdij-Hartzema, 1955 ; Maxwell, Ross, Plummer, and Sigg, 1957).

In order to see whether proof of an increase in circulating adrenaline could be obtained, direct estimations were carried out of the adrenaline concentration in the plasma of rabbits during the first 90 min. after an injection of reserpine. The rabbit was chosen because sufficient volumes of blood can be collected from a cut in the ear vein without restraining or anaesthetizing the animal. Further, in our experience, the medullary amines are more rapidly mobilized by reserpine from the adrenals of rabbits than from those of other laboratory animals. The disadvantage of the rabbit is the high 5-hydroxytryptamine (5-HT) content of its blood which greatly complicates the assay.

METHODS

Rabbits of an average weight of 2.9 kg. were used. The region of the marginal ear vein was shaved 1 or 2 days before the experiment. In order to collect blood, heparin (400 i.u./kg.) was injected into one ear vein and soon afterwards a small cut was made across the vein. The escaping blood was collected into an ice-cooled centrifuge tube containing 200 i.u. heparin. When 9 to 17 ml. of blood had been obtained, the haemorrhage was stopped by placing a light aluminium clip across the puncture. Reserpine was then injected into the vein of the other ear. Between 35 and 90 min. later, a second blood sample was collected by removing the clip and wiping the region of the puncture so that blood was again flowing freely from the vessel. With one exception, difficulty in obtaining blood owing to vasoconstriction was only experienced when iproniazid had been administered before the reserpine. Thus, after iproniazid and reserpine, the time required to obtain 6 ml. plasma (10 ml. blood) averaged 25 min., whereas the corresponding collection time after reserpine only was

4.8 min. In these experiments, and in one animal not given iproniazid, the ear had to be bathed repeatedly in warm saline in order to accelerate the flow.

The reserpine was given as Serpasil (Ciba) in doses of 1 to 2.3 mg./kg. Iproniazid (100 mg./kg. intravenously) was given either 1 or 14 hr. before the reserpine. Whereas rabbits not given any drugs showed no ill effects from the bleeding, all rabbits bled and injected with reserpine became comatose a few hours after the experiments and died in the course of the next 48 hr.

The cooled blood was centrifuged for 15 min. at 3,500 rev./min. and the plasma (usually between 6 and 9 ml.) was stirred into 6 vol. of cooled acid ethanol (0.1 ml. conc. HCl in 100 ml. ethanol). The further treatment of the alcoholic extract, the application to paper for separation of the amines by chromatography and the elution procedure were as previously described (Vogt, 1952).

The eluate of the noradrenaline region was tested on the blood pressure of the pithed rat. Rats of about 220 g. weight were injected subcutaneously with 1 to 2 mg. of atropine sulphate and deeply anaesthetized with ether. A tracheal cannula was then inserted and the central nervous system destroyed by introducing a wire (14 SWG) into one orbit, and passing it through the foramen magnum into the spinal canal (Shipley and Tilden, 1947). Artificial respiration was started immediately after the pithing. Such rats have a blood pressure between 50 and 60 mm. Hg and respond to the injection of 1 ng. of noradrenaline.

Adrenaline assays were carried out on the rat uterus stimulated by carbachol (Gaddum and Lembeck, 1949). On account of the large amounts of 5-HT present in the samples a special technique was required. The R_F value of 5-HT is only very slightly greater than that of adrenaline, so that eluates of the adrenaline region contain all the 5-HT recovered from the extracts. Before attempting the assay of adrenaline, the 5-HT equivalent of an aliquot of the eluate was determined on the rat uterus. Then a quantity of lysergic acid diethylamide (LSD) sufficient to abolish the effect of the highest 5-HT equivalent found in the eluates was added to the bath (doses of 0.15 to 1 μg. LSD were needed for a 2 ml. bath; they were left in the bath for 10, or occasionally 20, min.). The adrenaline was then assayed against standard solutions to which as much 5-HT had been added as had been found in the eluates. 0.05 or 0.1 ml. eluate corresponded to 1 ml. original plasma. Whenever adrenaline-like activity was found, an aliquot of the eluate was heated in a sealed tube at *p*H 8 to check for heat lability of the active substance.

RESULTS

Adrenaline.—It is known that the administration of reserpine to animals, previously treated with the amine oxidase inhibitor iproniazid, causes excitement and increased sympathetic activity, and that, in cats and dogs, iproniazid converts the depressor effect of reserpine into a pressor response (Brodie, Pletscher, and Shore, 1956; Chessin, Dubnick, Kramer, and Scott, 1956; Besendorf and Pletscher, 1956). The first experiments were carried out with this combination of drugs in the belief that the destruction of circulating adrenaline might thus be retarded (see Table I, rabbits 1 to 3).

TABLE I

ADRENALINE CONCENTRATION IN THE PLASMA OF RABBITS BEFORE AND AFTER AN INTRAVENOUS INJECTION OF RESERPINE

The ear vessels of rabbit No. 7 had to be dilated by bathing in warm saline during the collection of the first blood sample (S_1). In rabbit No. 1, S_1 was obtained before giving iproniazid, but in rabbits Nos. 2 and 3, S_1 was measured after iproniazid.

No. of Rabbit	Reserpine (mg./kg.)	Iproniazid Before Reserpine	Adrenaline μg./l. Plasma S_1 (Before Reserpine)	Adrenaline μg./l. Plasma S_2 (After Reserpine)	Adrenaline % Rise	Interval between Reserpine and S_2 (min.)
1	1·0	1 hr. before	<0·2	1·2	>500	43
2	2·2	14 hr. before	<0·8	3·0	>260	55
3	1·0	1 hr. before	1·3	2·0	54	80
4	2·3	None	0·1	1·2	1,100	38
5	2·3	,,	0·2	1·1	450	40
6	2·3	,,	<0·05	0·2	>300	40
7	2·2	,,	4·0	10·0	150	58
8	2·1	,,	0·8	1·6	100	97
9	None	,,	0·3	0·4	33	35
10	,,	,,	1·6	2·0	25	78

There was, in all three instances, an increase in circulating adrenaline after the reserpine. This was independent of the fact that rabbits Nos. 1 and 3, which had reserpine 1 hr. after iproniazid, were sedated, whereas rabbit No. 2, with a 14 hr. interval between the two drugs, was excited during the collection of sample 2. The experiments were, however, considered unsatisfactory because the intense vasoconstriction of the ear vessels slowed down the collection of the second samples and necessitated frequent bathing of the ear in warm saline and therefore much handling of the rabbit; this in itself might have increased the blood adrenaline.

The next five rabbits were treated with reserpine only, and these experiments were much more satisfactory. There was little difference in the collection time of the blood samples before and after reserpine, bathing of the ear with warm saline was not usually required, and the rabbits appeared indifferent to the procedure. All five rabbits (Table I, Nos. 4 to 8) had large increases in the adrenaline concentration of the blood after reserpine. The % increases appeared to be greater when the interval

between injection and sampling was short, but this may have been accidental, particularly since the absolute increases in circulating adrenaline did not reflect the same trend. It will be noted that the adrenaline concentrations before injecting the drug were very variable; rabbit No. 7, which had an abnormally high initial concentration, had constricted ear vessels and required bathing to obtain the requisite amount of blood. After reserpine the ear vessels were dilated, and the rabbit was sedated, easy to bleed, and no further warming of the ear was required. In spite of this, the adrenaline concentration was 2.5 times higher than before.

There was, however, one objection which could be raised against attributing the rise in circulating adrenaline to the action of reserpine. Haemorrhage is a physiological stimulus of the sympathetic system; thus it was conceivable that the blood loss connected with taking the first sample and not the drug was the cause of the increase in circulating adrenaline. Two experiments were therefore carried out on rabbits (Table I, Nos. 9 and 10) of the same size as those used before. Two blood samples were taken, the time allowed between the two samples covering the same range as the interval in the experiments with reserpine. The adrenaline content of sample 2 was, indeed, slightly higher than that of sample 1, but the increase was very much smaller than that following an injection of reserpine.

Noradrenaline.—Assays of noradrenaline were attempted in all plasma samples, and very small pressor effects were seen in the pithed rat when volumes of eluate equivalent to 2 to 3 ml. of plasma were injected. Assuming that these effects were due to noradrenaline, they indicated concentrations between 0.3 and 1 μg./l. plasma, and there were no consistent differences between samples obtained before and after reserpine. These quantities were too near the threshold of the method for tests of specificity to be carried out. It was, however, ascertained that the eluate did not mask the action of added noradrenaline, so that the figures should represent the upper limits of the real noradrenaline concentration, errors due to incomplete recovery having been shown to be small (Holzbauer and Vogt, 1954).

Discussion

The rise in circulating adrenaline seen after an injection of reserpine confirms the view that the loss in amines found in adrenals and ganglia is caused by a release from the tissue and not by an inhibition of synthesis. In this respect, there is complete parallelism with the effect of reserpine on 5-HT: synthesis of 5-HT is not impaired in tissue homogenates from reserpinized rabbits (Kuntzman, Udenfriend, Tomich, Brodie, and Shore, 1956), and its urinary metabolites rise to 4 or 5 times the normal value in dogs injected with reserpine (Shore, Silver, and Brodie, 1955). The noradrenaline levels in rabbit plasma were too near the threshold of the method to yield information on any changes that might have taken place. The upper limit of 1 μg./l. is very much lower than the figures reported in man by Burger (1957), who employed a fluorimetric method using condensation with ethylene diamine for his estimations. Assuming that the method was specific enough to estimate noradrenaline only, the fall in amine concentration after sedation by reserpine in Burger's patients might be explained on the assumption that mobilization of amines by reserpine requires larger doses than those used for sedation in psychiatric work.

Except for one rabbit (Table I, No. 6) the adrenaline concentration before reserpine was higher than that of completely resting rabbits, which, according to Armin and Grant (1955), should have values below 0.01 μg./l. This is easily explained by the amount of handling to which the rabbits were subjected.

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