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THE CONCENTRATION OF ADRENALINE IN THE PERIPHERAL BLOOD DURING INSULIN HYPOGLYCAEMIA

BY

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An increase in circulating adrenaline after insulin administration has been shown in the cat and the rabbit by indirect methods based on signs of stimulation of organs which had been deprived of their sympathetic nerve supply (Cannon, McIver, and Bliss, 1924; Abe, 1924). An increased secretion of adrenaline into the adrenal vein was demonstrated during insulin hypoglycaemia in the dog by Houssay, Lewis, and Molinelli (1924). Poll (1925) showed that the adrenal medulla contains but little chromaffin substance after the injection of a large dose of insulin. More recently, quantitative estimations of the amines remaining in the adrenal medulla after injection of insulin have been carried out by several authors (e.g., Burn, Hutcheon, and Parker, 1950; West, 1951; Outschoorn, 1952); a fall was invariably reported. Euler and Luft (1952) have shown that the urinary excretion of adrenaline rises in man after an injection of insulin. The reverse phenomenon, namely a fall in the amount of adrenaline secreted into the adrenal vein of the cat made hyperglycaemic by infusion of glucose, was demonstrated by Dunér (1953).

In contrast to these findings, Weil-Malherbe (1953) and Weil-Malherbe and Bone (1952), using a fluorimetric method of adrenaline estimation in human blood, report a decrease in the adrenaline content of peripheral blood after intravenous insulin. This finding seemed difficult to reconcile with current physiological concepts. Since no direct estimations of the adrenaline content of peripheral blood after the injection of insulin have been made by other methods, it was decided to attempt such estimations by a technique which left very little doubt whether the estimated compound was really adrenaline.

METHODS

The experiments were carried out on two dogs and one human volunteer after an overnight fast.

Eight experiments were performed on the dogs with doses of insulin ranging from 0.2 to 2.1 u./kg.; 0.24 u./kg. was used in the one experiment carried out on the human subject. The insulin was injected intravenously shortly after the collection of a control sample of blood.

Collection of Blood.—No difficulty was experienced in obtaining blood samples free of 5-hydroxytryptamine (see later) in man by collecting blood from the brachial vein into a siliconed syringe containing 500 u. heparin. The same technique was successful in the dog, when single blood samples were drawn from one of the leg veins. In those experiments, however, in which a series of blood samples were required at precise time intervals, a technique had to be chosen which avoided frequent puncturing of the veins and even the slightest degree of clotting. Further, the collection had to be carried out without any emotional disturbance of the dog. The following procedure fulfilled these requirements. The dogs were trained to stand in a Pavlov stand. On the day of the experiment, some 0.1% cinchocaine solution was injected into and under the skin covering one of the saphenous or cephalic veins. A cannula consisting of a bent piece of polythene tubing (inner diameter 1 mm.) carrying several holes near its tip was used. Its end was connected by means of a syringe needle to a long piece of soft rubber tubing leading to a 100 ml. burette clamped about 1 m. above the dog. The whole system was filled with sterile 0.9% NaCl solution containing heparin (500 u./100 ml.). A screw clip on the rubber tubing controlled the flow from the burette. The cannula was inserted into the vein by threading it through a serum needle which had been introduced, pointing distally, into the vein. The serum needle was withdrawn as soon as the polythene cannula was in place. The cannula was secured in position by a swab of gauze and strips of adhesive plaster wound around the leg. To avoid clotting it was essential that the heparinized saline should flow through the polythene cannula while it was being introduced into the vein. Once the cannula was in position, the flow was adjusted to not less than 0.5 ml./min. With this arrangement, samples of blood were obtained by

disconnecting the syringe needle from the polythene tubing, discarding the first millilitres of blood, and collecting 12–15 ml. blood into a siliconed centrifuge tube which contained 600 u. of heparin in 0.1 ml. saline. Slight massage of the foot was sometimes used to accelerate the flow, but this was unnecessary when the blood escaped from the vein at some pressure, as was frequently the case. When enough blood had been collected, the needle leading to the infusion burette was replaced into the polythene tube, the tube was flushed with saline and the slow infusion resumed. The long rubber tubing allowed for a certain amount of movement of the dog's leg without danger to the polythene cannula. Excitement of the dog during sample collection was not noticeable. Burette, tubing, needles, and polythene cannula were all sterilized before use.

The first blood sample was collected 5–10 min. after introduction of the cannula; the insulin was then injected through the rubber tubing, and five further blood samples were collected at intervals. The earliest samples were taken 5 min., and the latest 210 min., after the injection.

Blood sugar estimations were carried out by the method of Hagedorn and Jensen (1923).

Extraction of Adrenaline and Noradrenaline.—The extraction process has been described earlier (Vogt, 1952a). Plasma samples of 5.5 ml. were obtained as rapidly as possible from the heparinized, cooled blood and extracted with acid alcohol. The extracts were purified, adrenaline and noradrenaline separated by paper chromatography, the amines eluted, the eluates evaporated to dryness and taken up in 0.55 ml. H_2O (tenfold concentration of the plasma).

Assays.—Adrenaline was assayed on the rat's uterus stimulated by carbachol (Gaddum and Lembeck, 1949). Before the finally adopted technique of blood collection had been developed, the eluates frequently contained a substance which stimulated the rat's uterus. Its position on the chromatogram, and the disappearance of its stimulating effect after the addition to the uterus bath of dihydroergotamine or lysergic acid diethylamide (Gaddum, 1953), make it extremely likely that this substance was 5-hydroxytryptamine (HT). Though assays of adrenaline in a mixture of adrenaline and HT may be carried out by using the antagonists dihydroergotamine and lysergic acid diethylamide, such assays are not satisfactory, because the mixture has frequently not precisely the same effect on the uterus as the same amount of adrenaline alone. They were thus not relied upon and the results of such assays are not included. The interference of very small quantities of HT can be avoided by choosing uteri which are not oestrous; these may be very sensitive to adrenaline and are fairly insensitive to HT.

It soon became obvious that the quantities of adrenaline occurring in blood were even smaller than had been suspected, and required extremely sensitive uteri for their estimation. Though such uteri (sensitive to 0.03–0.2 ng. adrenaline in a 2 ml. bath) are occasionally found, they are rare, and a method of sensitizing the uterine tissue was therefore sometimes resorted to. This consisted in adding small concentrations of the adrenaline antagonist (see Jang, 1941) dibenzyline (SKF 688A or *N*-phenoxy*iso*propyl-*N*-benzyl-β-chloroethylamine HCl) to the bath; doses of between 0.1 and 0.5 μg. were introduced into the 2 ml. bath for a period of 10 min. and then washed out; after this treatment sensitization to the desired degree was usually obtained. If the lowest concentration does not have the desired effect it is best to increase the dose of dibenzyline very gradually; the responses to carbachol are otherwise likely to become depressed and irregular. Since the latent period of the contraction is prolonged by the drug, it is also advisable to leave the carbachol in the organ bath for a period which is between 10 and 15 sec. longer than the normal time of 40 sec.

No inhibition of the carbachol contraction of the uterus was accepted as being due to adrenaline unless the action disappeared on heating the solution in a stoppered flask for 10 min. in a boiling water bath in the presence of $NaHCO_3$. If no adrenaline was found, a test was made to ensure that no "masking" substances were present by adding known amounts of adrenaline to the eluate and comparing the effect of the mixture with the effect of adrenaline dissolved in saline. These criteria were fulfilled for all the figures given under "Results."

Recovery—In an earlier paper (Vogt, 1952a) recovery had been tested by adding 0.2 μg. of adrenaline and noradrenaline per ml. plasma and extracting 5 ml. of the mixture. The chromatographic procedures had been tested by adding a total of 5 ng. adrenaline per sample. These amounts were too large to be satisfactory for the present work, since the largest amounts of adrenaline found were usually of the order of 0.5–2.0 ng./ml. plasma. Recovery experiments were therefore carried out in which adrenaline and noradrenaline were added to 5.5 ml. plasma, to produce final concentrations, per ml., of 2 and 0.4 ng. adrenaline and of 4 and 1 ng. noradrenaline. Recovery at both dose levels was complete within the limits of the error of the assay; losses, therefore, cannot have amounted to more than 30%. The plasma used for these recoveries did not contain amines in concentrations detected by our methods.

Results

Fig. 1 shows the results of four experiments, carried out on one dog, and in which all blood samples were free of interfering amounts of 5-hydroxytryptamine and could be satisfactorily assayed. In three experiments the concentration of adrenaline in the plasma before the injection of insulin lay between 0.04 and 0.25 μg./l. In the fourth ,no adrenaline (<0.06 μg./l. was found, but the adrenaline concentration rose to 0.36 μg./l. 5 min. after the injection of 1.8 u./kg. of insulin.

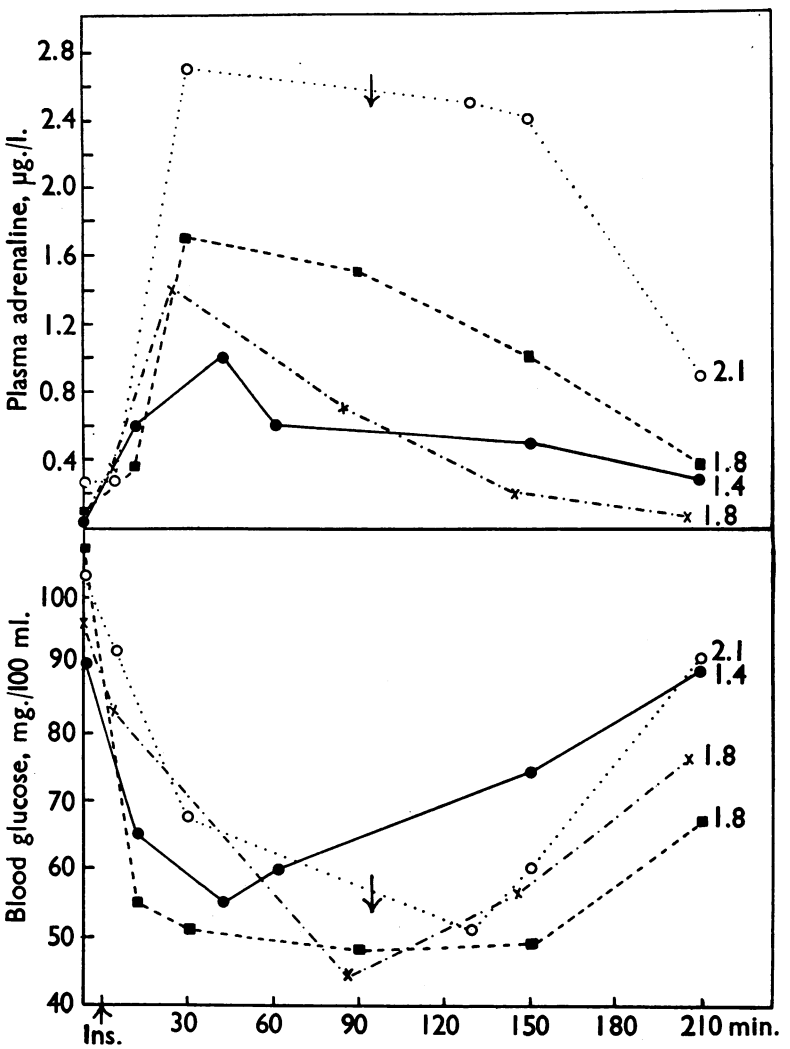

FIG. 1.—Effect of an intravenous injection of insulin on the concentration of adrenaline in plasma. Four experiments on the same dog. Ordinate, upper half, plasma adrenaline in µg./l. Lower half, blood glucose in mg./100 ml. Abscissa, time in min. At the arrow marked 'Ins.' injection of insulin; doses in u./kg. body weight at the end of the curves. At the arrows pointing down, 1 g. glucose was injected intravenously (experiment marked O · · · · · O).

In this dog, the highest levels of plasma adrenaline were attained between 25 and 43 min. after the insulin; in the course of 210 min. the concentration returned towards or to the pre-injection figure. The greatest adrenaline concentrations were observed in the experiment in which the largest dose of insulin was given, despite the fact that the full effect of the insulin was not allowed to develop: the dog was given 1 g. of glucose intravenously 100 min. after the insulin in order to counteract muscular weakness which the animal was showing as a result of the hypoglycaemia.

Experiments carried out on another dog with smaller doses of insulin (0.2–0.8 u./kg.) agreed with the findings of Fig. 1. The data, however, are incomplete, since only three or four out of the six samples collected in each of the four experiments were suitable for assay. In one sample of plasma taken before the injection of insulin, no adrenaline was detectable (<0.5 µg./l.). In two samples obtained 5 min. after the injection, the blood glucose had fallen and the adrenaline had risen sharply to 4.0 and 6.4 µg./l. plasma. Recovery to control levels was quicker than after the larger doses of insulin.

Table I shows the results of a similar experiment conducted in a healthy human subject. The adrenaline concentration in plasma from the cubital vein was <0.06 µg./l. before insulin was injected and rose rapidly to reach 1.8 µg./l. in 45 min. At this time there was some muscular weakness and profuse sweating, and the experiment was terminated by giving glucose. Apart from the smaller dose of insulin required in man to produce the same fall in blood glucose and the same concentration of circulating adrenaline as in the dog, the responses of man and dog were essentially the same.

Noradrenaline estimations were attempted in all the experiments, but none was found. The eluates

TABLE I

EFFECT OF AN INTRAVENOUS INJECTION OF INSULIN (0·24 U./KG.) ON THE CONCENTRATION OF ADRENALINE IN PLASMA OF A HUMAN SUBJECT

Time after Injecting Insulin (min.)	Adrenaline in Plasma (µg./l.)	Blood Glucose (mg./100 ml.)
−1	<0·06	121
5	0·5	75
45	1·8	55

of the noradrenaline paper-strip had a slight pressor effect, equivalent to 0.5 ng. and, occasionally, 1 ng. noradrenaline per ml. plasma, but this effect was heat stable under conditions when noradrenaline, added to the same eluates, was not. It was shown under "Methods" that noradrenaline, added to plasma to produce a concentration of 1 μg./l., was detectable by the assay procedure, so that the concentration of noradrenaline in the plasma of dog and man must have been less than 1 μg./l.

Discussion

The foregoing experiments show that, both in man and in the dog, the concentration of plasma adrenaline rises in the venous blood when hypoglycaemia is produced by insulin. After insulin, the content of adrenaline in the plasma ranged from 0.25 to 6.4 μg./l., and was thus of the same magnitude as that of the 1 to 6 μg./l. found in arterial plasma of rats during a surgical operation under ether (Vogt, 1952b). These observations are difficult to reconcile with those of Weil-Malherbe (1953) and Weil-Malherbe and Bone (1952), who used a chemical method and reported a fall in blood adrenaline in hypoglycaemia. Another discrepancy lies in the "normal" figures for sympathomimetic amines shown by the two methods. According to biological assay, the adrenaline concentration in plasma of the undisturbed conscious dog lay invariably below 0.25 μg./l. and was sometimes less than a quarter of this figure. The one figure obtained in man was <0.06 μg./l. The noradrenaline content of plasma was below the threshold of the biological method, which lies at 1 μg./l. Corresponding figures reported by Weil-Malherbe and Bone (1953) are about 1 μg. adrenaline and 5 μg. noradrenaline per l. plasma and are thus at least five times as high as those determined biologically. Since we found the recovery of added amines at these levels perfectly satisfactory, the lower figures can hardly be caused by losses during the extraction process. It is at least conceivable that the chemical method estimates other compounds in addition to the sympathomimetic amines.

Summary

1. The changes in the adrenaline concentration of peripheral venous blood produced by an injection of insulin were followed in the dog and in a human subject.

2. There was invariably a rise in the adrenaline content of the plasma which started a few minutes after the intravenous injection of insulin and lasted for several hours. The rise increased when the dose of insulin was raised. The concentrations observed ranged from 0.25 to 6.4 μg./l. plasma.

3. The method employed for the assay of adrenaline permitted the recovery of adrenaline added to plasma in concentrations of 0.4–2 μg./l.; it was quite adequate for the estimation of the adrenaline concentrations which occurred after the injection of insulin, but not always for the detection of adrenaline before insulin was given; in these samples the concentrations, when above threshold, had a mean of 0.13 μg./l.

4. No noradrenaline was found in any of the plasma samples either before or after the injection of insulin. Since the method allowed the detection of noradrenaline added to plasma in a concentration of 1 μg./l., it follows that the noradrenaline content of all samples was below that figure.

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