Brit. J. Pharmacol. (1951), **6**, 289.

INSULIN AND THE SUPRARENAL GLAND OF THE RABBIT

BY

G. B. WEST

From the Pharmacological Laboratory, University of St. Andrews, Medical School, Dundee

(Received February 2, 1951)

When animals are injected with insulin in amounts sufficient to produce the symptoms of hypoglycaemia, a discharge of adrenaline and/or *nor*adrenaline from the suprarenal medulla occurs which helps to restore the normal blood sugar. The discharge does not occur if the splanchnic nerve is severed, nor does it occur when insulin is injected into the suprarenal artery. Burn, Hutcheon, and Parker (1950b) found that insulin treatment in rats resulted in a decline in the total activity of suprarenal gland extracts and in the percentage of adrenaline in them. They suggested that *nor*adrenaline is the precursor of adrenaline in the rat, and that after several hours' depletion of the medulla the supply of *nor*adrenaline is restored more rapidly than it can be methylated to form adrenaline. A similar finding has already been reported in the cat, after electrical stimulation of the splanchnic nerve (Bülbring and Burn, 1949a).

Only minute quantities of *nor*adrenaline are found in extracts of normal suprarenal glands of rabbits. When the plasma in the adrenal vein before and after stimulation of the splanchnic nerve is analysed, small quantities of *nor*adrenaline can be detected in samples collected soon after stimulation commences; continued stimulation results in its disappearance, only adrenaline then being detected (West, 1950). It seemed of interest, therefore, to investigate the content of the gland of the intact unanaesthetized animal under insulin treatment.

METHODS

For this study 66 rabbits of both sexes were used. As far as possible each set of results was obtained with one rabbit weighing under 1 kg. and one rabbit weighing 1.5–2.5 kg. The weights are important, as younger rabbits are more sensitive to insulin than are older ones. They were kept without food overnight. In the morning, blood from the ear vein was removed for blood-sugar determinations (Hagedorn and Jensen method), and then the injections were given. The doses of insulin were 2, 3.15, or 5 units per kg. subcutaneously; these were sufficient to produce symptoms of hypoglycaemia. With the higher dose levels symptoms were sometimes severe; these animals were either given intravenous injections of glucose or killed.

At a given time after the insulin injection, blood was again removed from the ear vein for sugar estimations. Immediately afterwards, the rabbits were killed by a blow on the head and bled out by cutting the carotid artery. The suprarenals were removed, dissected free from the capsules, weighed, and ground in a mortar with sand and 10 ml. 0.1 N–HCl/g. (Holton, 1949). The acid extracts were filtered, and the filtrates assayed for their adrenaline

and *nor*adrenaline contents by the action on the blood pressure and normal nictitating membrane of a spinal cat (Burn, Hutcheon, and Parker, 1950a). Total activity was usually determined in the isolated rabbit ileum. In certain experiments the extracts were assayed on the isolated rectum of a chick, the isolated uterus from a non-pregnant rat in dioestrus, or the uterus of a non-pregnant spinal cat. Assays were always completed within 24 hours from the time of killing the animals. Solutions of *l*-adrenaline and *l-nor*adrenaline were prepared in 0.01 N-HCl.

In a few experiments the glands were ground in a mortar with 0.25–0.5 ml. 0.1 N-HCl and then centrifuged. A measured volume of the clear supernatant was used for chromatographic identification and determination (James, 1948; Euler and Hamberg, 1949). An ascending column was used with a butanol–acetic acid–water solvent. For developing, 0.44 per cent potassium ferricyanide solution in phosphate buffer *p*H 7.8 was used. The remainder of the supernatant was diluted and used for the biological determination, as described above.

RESULTS

Changes in total activity.—The changes in total activity for all experiments are shown in Table I. The mean control value of 485±131 μg. adrenaline per g. fresh tissue was obtained from 24 rabbits. There were slight variations in each group

TABLE I

THE INFLUENCE OF INSULIN ON THE ADRENALINE CONTENT OF THE SUPRARENAL GLANDS OF THE RABBIT

Dose of insulin (units/kg.)	Adrenaline content (μg./g.) at various times after insulin							
	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.	6 hr.	8 hr.	12 hr.
2.00	821	648	220	173	208	473	740	640
3.15	1,044*	922	355	170	189	450	735	750
5.00	1,117†	700	274‡	95‡	235‡	—	—	—
Mean ..	994	757	283	145	211	462	737	695

Control value of adrenaline in rabbit adrenals = 485 ± 131 μg./g.

* 50 μg. *nor*adrenaline/g. also found. † 41 μg. *nor*adrenaline/g. also found. ‡ I.V. glucose needed.

after insulin, and usually the value for the younger animal was lower than that recorded for the older one. During the first two hours the adrenaline content of the gland rose significantly, and it was only during this period that *nor*adrenaline was detected in certain extracts and estimated in amounts representing less than 6 per cent of the total amount. The adrenaline content dropped sharply during the next two hours, reaching maximum depletion at about four hours. From the biological tests there was no doubt at this stage that only adrenaline was present (see Fig. 1); a negative value was also obtained in the chromatographic experiments, indicating that less than 5 per cent *nor*adrenaline was present in the total mixture. By the sixth hour the values had reached the control level and only adrenaline was detected. The mean results are plotted in Curve A (Fig. 2). It will be noted that intravenous glucose was only necessary two hours after the high dose of insulin.

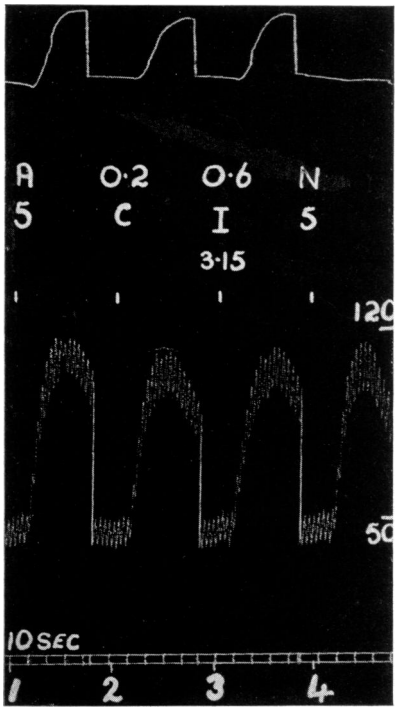

FIG. 1

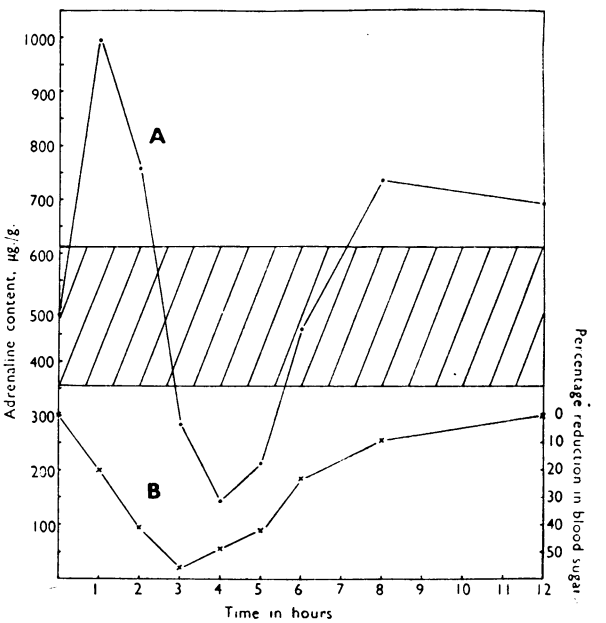

FIG. 2

FIG. 1.—Spinal cat. Normal nictitating membrane and blood pressure. (1) 5 µg. *l*-adrenaline; (2) 0.2 ml. extract of suprarenal glands of control rabbit; (3) 0.6 ml. extract of suprarenal glands of rabbit given 3.15 units insulin/kg. 4 hours previously; (4) 5 µg. *l-nor*adrenaline.

FIG. 2.—Curve A shows mean change in adrenaline content of rabbit suprarenals after insulin. Ordinates (on left) µg./g. Abscissa time in hours. Curve B shows mean percentage blood-sugar reduction (ordinate on right). Note the initial increase in adrenaline content in the first two hours. Values above or below the shaded area are significant.

Changes in blood sugar.—The percentage reduction in blood-sugar content was determined in each experiment, and the mean results for each group are shown in Table II. The maximum decline occurred at about three hours and recovery had almost taken place by eight hours (Fig. 2, Curve B).

TABLE II

THE EFFECT OF INSULIN ON THE BLOOD SUGAR OF RABBITS

Dose of insulin (units/kg.)	Percentage blood-sugar reduction at various times after insulin							
	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.	6 hr.	8 hr.	12 hr.
2.00	20	48	54	41	36	18	3	1
3.15	18	41	55	57	49	29	15	2
5.00	22	49	58	—	—	—	—	—
Mean ..	20	46	56	49	42	23	9	0

Discussion

The results show in the first place the change in total activity of the suprarenal medulla of the rabbit after injections of insulin in sufficient amounts to cause symptoms of hypoglycaemia. In the first two hours, while the blood sugar is steadily falling, a significant increase in activity occurs; the gland is calling on its reserves, which appear to be quite extensive. A similar increase in the total activity of the stimulated gland compared with that of the unstimulated gland of the opposite side was previously noted in the eviscerated animal under urethane anaesthesia (West, 1950) with the splanchnic nerve stimulated electrically for periods up to two hours. After the initial increase a rapid decline in total activity occurs, minimal values being recorded four hours after the insulin injection, approximately one hour after the maximal percentage blood-sugar reduction. At this stage the gland is being quickly exhausted in response to the hypoglycaemia, and total activity falls to less than 30 per cent of that originally present. By six hours recovery has taken place; in fact, the process of catechol formation appears to be proceeding at a fast rate, for total activity values obtained at eight and twelve hours are raised above the control value, despite the fact that the blood sugar is still below the normal fasting level (mean value 85 mg. per 100 ml.).

The results show in the second place the presence of small quantities of *nor*-adrenaline in the suprarenal gland about one hour after the injection of insulin. This is the only occasion when *nor*adrenaline has been detected. In the anaesthetized animal (West, 1950) it was shown that *nor*adrenaline was released into the plasma in the suprarenal vein only during the first ten minutes of continuous electrical stimulation of the splanchnic nerve, and that adrenaline was present in large quantities at all times.

Evidence for the assumption that *nor*adrenaline is the precursor of adrenaline has been obtained by Bülbring (1949), who showed that minced suprarenal tissue from cat and from dog was able to convert *nor*adrenaline to adrenaline, provided that adenosine triphosphate was present. Bülbring and Burn (1949b) also showed that *nor*adrenaline is converted into adrenaline in the isolated perfused suprarenal gland of the dog.

Under the influence of insulin (large or small doses) the suprarenal gland of the rabbit calls on its reserves and the output of adrenaline is increased, traces of *nor*-adrenaline then appearing. As the gland becomes exhausted, the work of replenishing the store of *nor*adrenaline in the medulla may proceed more slowly than the process of methylation, and only adrenaline is found. It may well be that *nor*adrenaline is not a precursor of adrenaline in the rabbit. This mechanism does not compare, therefore, with that seen in the cat, dog, or rat. In these animals prolonged stimulation results in a greater outpouring of *nor*adrenaline as time proceeds, so that there is a fall in total activity, and also a fall in the percentage of adrenaline, in the gland. Nevertheless, Burn, Hutcheon, and Parker (1950b) found in rats that two hours after the injection of insulin the total activity of the gland had decreased but the percentage of adrenaline in the mixture had risen slightly (indicating increased methylation). It is possible that a shorter time interval might have disclosed an increase in total activity similar to that seen in the rabbit.

SUMMARY

1. When insulin is injected in sufficient amount the activity present in the suprarenal medulla of the rabbit first increases and then declines. The increase occurs during the first two hours after injection, and the fall is evident after three hours. The minimum value is reached at about four hours and recovery has taken place by six hours.

2. During the first two hours after injection of insulin, *nor*adrenaline in small amounts may appear in the extract of the suprarenal gland. At all other times the gland contains only adrenaline.

3. If *nor*adrenaline is a precursor of adrenaline in the suprarenal gland of the rabbit, then methylation to form adrenaline occurs rapidly, even in the exhausted gland.

I wish to record my thanks to Professor R. B. Hunter for his helpful advice, and to Mr. D. M. Shepherd of this Department for carrying out the chromatographic studies.

REFERENCES

Bülbring, E. (1949). *Brit. J. Pharmacol.*, **4**, 234.
Bülbring, E., and Burn, J. H. (1949a). *Brit. J. Pharmacol.*, **4**, 202.
Bülbring, E., and Burn, J. H. (1949b). *Brit. J. Pharmacol.*, **4**, 245.
Burn, J. H., Hutcheon, D. E., and Parker, R. H. O. (1950a). *Brit. J. Pharmacol.*, **5**, 142.
Burn, J. H., Hutcheon, D. E., and Parker, R. H. O. (1950b). *Brit. J. Pharmacol.*, **5**, 417.
Euler, U. S. v., and Hamberg, U. (1949). *Nature, Lond.*, **163**, 642.
Holton, P. (1949). *J. Physiol.*, **108**, 525.
James, W. O. (1948). *Nature, Lond.*, **161**, 851.
West, G. B. (1950). *Brit. J. Pharmacol.*, **5**, 542.