Increased Adrenaline Production Following Administration of 2-Deoxy-D-Glucose in the Rat.* (26394)

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Following the observation(1) that administration of 2-Deoxy-D-Glucose (2-DG) to the eviscerated-nephrectomized rabbit decreases the amount of glucose required to maintain a constant blood sugar level, data have been presented to support the hypothesis that 2-DG acts as a metabolic blocking agent inhibiting glucose utilization. Thus, in spite of the increased blood glucose level following 2-DG in the rat(2), dog(3) and man (4), a condition results which has been termed "cellular hypoglycemia" or cellular glucopenia(2). Since the blood sugar level can affect secretion of adrenal medullary hormones we have tested the effect of 2-DG on secretion of adrenaline and noradrenaline in the intact rat. When positive results were found, additional experiments were carried out in rats with denervated adrenal glands.

Material and methods. 2-DG was obtained from the Aldrich Chemical Co., Milwaukee, Wis., through the courtesy of the Cancer Chemotherapy National Service Center, NIH, Bethesda. In all instances 2-DG was administered subcutaneously (s.c.) in water solution (50 mg/ml). In control animals a corresponding quantity of distilled water was injected. Regular insulin (Insulin Vitrum) was injected undiluted s.c.

Blood glucose concentrations were determined colorimetrically using condensation with O-toluidine(5); in this procedure 2-DG gives only one-tenth of the color produced by equal quantities of glucose within the range of 50-1000 mg %. Tail vein blood was used.

Adrenaline and noradrenaline production were judged by urinary excretion of free catechol amines, estimated fluorimetrically(6). To obtain sufficient amounts, urine was collected over 8-12 hours from groups of 2-4 rats in metabolic cages. At the end of each experiment the catecholamine content of the adrenals was estimated by the same procedure after extraction with 3% trichloracetic acid but without absorption on alumina.

The adrenal glands were denervated either by spinal cord transsection at the level of 0:7, or by division of the splanchnic nerves immediately below the diaphragm *via* the abdominal approach. Intraperitoneal barbiturate anaesthesia of short duration (Citodon-Na, Leo) was used. The rats were killed by decapitation.

Inbred male rats, 175-275 g, maintained on a diet of known composition, were used. During the acute experiment no food was given, but free access to water was allowed.

Results. It was clearly shown that 2-DG, when administered in sufficiently high doses to maintain an elevated blood sugar level for a longer period of time, produces a marked increase in adrenaline secretion. Thus, when 2-DG was given in a dose of 50 mg per 100 g body weight twice over an 11 hour period, urinary adrenaline increased about 40 times (Table I); urinary noradrenaline excretion was doubled. Secretion of adrenal medullary hormones apparently was intense enough to make resynthesis lag behind, as revealed by pronounced lowering of adrenaline content of the adrenal glands. The changes in urinary and adrenal catecholamines after 2-DG closely resembled those obtained after insulin-induced hypoglycemia over the same period (Table I). Following administration of 2-DG in smaller dosage, both the increase in catecholamine secretion and the hyperglycemic response were less pronounced.

After adrenal denervation by transsection of the spinal cord, urinary adrenaline remained normal, but a minor decrease in noradrenaline excretion probably occurred. In these rats, urinary catecholamine excretion

* This work was supported by grants from Swedish Medical Research Council, Magn. Bergwall Foundation and The Åhlén Foundation.

TABLE I. Adrenaline and Noradrenaline Content of Adrenals and Urine after Subcutaneous Administration of 2-DG and Insulin in Intact and Spinal Rat.

	Adrenals			Urine		
	No. of rats†	Noradrenaline, μg/kg b.w.	Adrenaline, μg/kg b.w.	No. of urine samples‡	Noradrenaline, ng*/kg/hr	Adrenaline, ng*/kg/hr
Normal controls	10	17.2 ± 1.82‖	149 ± 6.7	5	73 ± 8.4	21 ± 5.1
2-DG						
25 mg × 1	4	7.8 ± .95	75 ± 10.8	1	118	153
50 mg × 1	4	11.0 ± 2.35	79 ± 21.0	1	111	367
50 mg × 2§	8	13.1 ± 2.39	32.3 ± 3.69	4	146 ± 19.1	806 ± 7.0
Insulin, 1 unit × 2§	6	15.7 ± 1.99	25.5 ± 7.32	4	197 ± 40.6	912 ± 90.3
Spinal controls	6	46.5 ± 3.95	188 ± 8.55	2	47–32	24–53
Spinal + 2-DG, 50 mg × 2§	7	43.4 ± 5.47	173 ± 9.70	2	33–42	22–53

* ng = .001 μg. † Both adrenals analyzed together. ‡ Urine from groups of 2-4 rats.
§ 5 hr interval between injections. ‖ Stand. error.

after injection of 2-DG did not change; blood glucose level probably increased (141 mg % ± 6.6, spinal control 103 mg % 2.9), although rather moderately as compared with the intact animal (264 mg% ± 13.6). Furthermore, 2-DG caused no change in adrenaline and noradrenaline content of the adrenals in the spinal rat; however, noradrenaline content was higher in the adrenals of the spinal controls as compared to normal rats. Thus, the effect of 2-DG on production of adrenaline and noradrenaline is mediated *via* a central nervous mechanism.

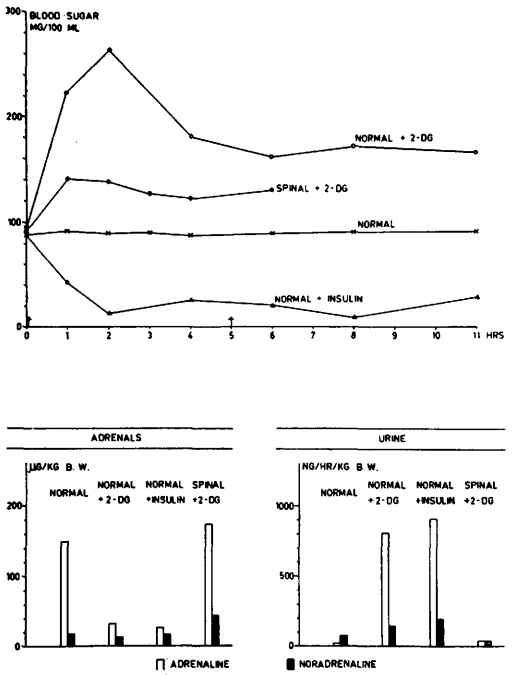

FIG. 1. Upper part: Blood sugar response evoked by 2-DG (50 mg/100 g body wt × 2) in intact and spinal rats as compared to untreated controls and normal rats given insulin (1 unit/100 g body wt × 2); ↑ denotes time of inj. (s.c.). Lower part: Adrenaline and noradrenaline in the adrenals and the urine from the same rats.

After unilateral transsection of the splanchnic nerves, observations similar to those in the spinal rats were made. Thus, after injection of 2-DG (50 mg per kg body weight × 2) the denervated adrenals contained normal amounts of adrenaline (155-156 μg per kg b.w.) while a marked decrease (to 27-32 μg) took place in the intact adrenal; the corresponding figures for noradrenaline were 45-55 μg and 27-33 μg, respectively.

Discussion. Although the symptoms appearing in animal and man after administration of 2-DG were noted by Landau *et al.*(2, 4) to be similar to those seen after insulin-induced hypoglycemia, he suggested that these phenomena were not related to adrenaline release(4). Working with anaesthetized rats Brown and Bachrach, however, could demonstrate that the hyperglycemic response to 2-DG in the intact animal was abolished by adrenal demedullation, and suggested that 2-DG is capable of stimulating adrenaline release(7). In the present studies, direct evidence supports this hypothesis. Adrenaline release of the order of magnitude seen after 2-DG has previously been found only after

convulsive doses of insulin in the rat, leading to almost total depletion of the adrenaline stored in the adrenal medulla(8). This has been confirmed in the present study. In comparison, such factors as work and hypoxia seem to cause only limited adrenaline release(8).

According to our findings, the adrenaline secretion evoked by 2-DG is the result of a central nervous stimulation. The presence of a centrally located receptor mechanism regulating adrenaline secretion was postulated in 1924 by Cannon(9) and Houssay (10), who demonstrated that the adrenaline release in hypoglycemic animals could be abolished by adrenal denervation. Dunér (11) in experiments in the cat gave some evidence that this center is located in the hypothalamus. He injected glucose locally in this area and found an inhibition of adrenaline secretion, which made him postulate a receptor mechanism sensitive to glucose. In the present studies, adrenaline release was evoked under conditions of hyperglycemia (after 2-DG) and hypoglycemia (after insulin). Blood glucose level *per se* is not the regulating factor. It seems likely that adrenaline release is linked to inhibition of glucose utilization caused by 2-DG(12). The hyperglycemia after 2-DG to a great extent depends on the release of adrenaline. That some increase in blood glucose level was still seen following 2-DG in rats after denervation of the adrenals might indicate that other factors are also involved. These studies will be enlarged, particularly in view of the report(7) that no change occurred in blood sugar level of adrenal demedullated rats after 2-DG. A pertinent question is whether 2-DG could lead to erroneously high values by interference with our procedure for blood glucose. This is unlikely, since a 2-DG level of 400 mg % would have been required to simulate an increase in blood glucose of 40 mg % as observed in spinal rats given 50 mg 2-DG per kg b.w. Further more, Landau and Lubs (2) found blood 2-DC values around 3 mg % 2 hours after s.c. administration of 40 mg of 2-DG per 100 g b.w. in the rat.

Summary. 2-deoxyglucose (2-DG) causes marked release of adrenaline from the adrenal medulla in the intact rat but not after adrenal denervation. The adrenaline released is to a great extent responsible for the hyperglycemia appearing after 2-DG, but other factors might also be involved. The inability of 2-DG to cause increased adrenaline secretion after denervation of the adrenal glands points to its action *via* a centrally located receptor mechanism regulating adrenaline secretion; this center seems not to be sensitive to the blood glucose level as such.

The authors are indebted to Drs. D. J. Ingle and M. B. Lipsett for valuable discussion and criticism.

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Received December 19, 1960. P.S.E.B.M., 1961, v106.