Estimation of Adrenomedullary Reserve by Infusion of 2-Deoxy-D-glucose

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ABSTRACT. Infusions of 2-deoxy-D-glucose (2-DG), in concentrations of 50 mg/kg body wt, produced a consistent and pronounced increase in urinary epinephrine output in subjects with intact adrenal glands. They also produced a significant rise in urinary epinephrine in patients with Addison's disease classified as idiopathic by clinical criteria, but not in those whose disease was considered tuberculous in origin, or in subjects with adrenalectomy or thoracolumbar sympathectomy. A rise in blood glucose has been demonstrated to correlate well with the increase in urinary epinephrine that follows 2-DG administration. Measurement of these substances affords a clinically useful means for evaluating the functional capacity of the adrenal medulla. (*J Clin Endocr* **26:** 37, 1966)

AN ESTIMATE of the ability of the adrenal medulla to produce epinephrine is of value in understanding the pathophysiology of such disorders as idiopathic spontaneous hypoglycemia of infancy (1–4), postural hypotension (4, 5), severe burns (6) and prolonged insulin hypoglycemia (7, 8).

Hypoglycemia induced by the administration of insulin was first shown to increase significantly the output of epinephrine in animals in 1924 by Cannon, McIver and Bliss (9), and Houssay, Molinelli and Lewis (10), and in man in 1952 by von Euler and Luft (11). Since then this means of stimulating the adrenal medulla has been used in many clinical studies.

Received July 2, 1965; accepted August 27, 1965.

This work was supported in part by Grant AM-07654 from the National Institute of Arthritis and Metabolic Diseases, USPHS. Dr. Wegienka and Dr. Grasso are Trainees under Grant T1 AM-5115-08 from the same institution. Patients were studied in the General Clinical Research Ward, maintained by Grant FR-79 from the Division of Research Facilities and Resources, USPHS.

The administration of 2-deoxy-D-glucose (2-DG) affords a means of interfering with carbohydrate metabolism under controlled conditions (12–21) by blocking the intracellular utilization of glucose, thereby inducing partial cellular glucopenia in the nervous system. This agent has been shown to increase the output of epinephrine from the intact adrenal medulla in the rat (15) and in man (12, 13).

We used this agent to estimate adrenal medullary epinephrine reserve by giving 2-DG to normal subjects and to patients with various endocrine disorders. Its usefulness for distinguishing between tuberculous and idiopathic Addison's disease was evaluated and found to afford a means of differentiating between the two.

Materials and Methods

The chemically pure form of 2-DG was dissolved in distilled water to make a 20% solution, passed through a millipore filter and stored in a sterile container under refrigeration at 4 C. Samples were tested regularly for possible bacterial contamination.

2-DG in a dose of 50 mg/kg body wt in 100 ml normal saline was given intravenously for the first 30 min of a 2½–3 hr infusion. Blood pressure and pulse rate were measured every 30 min for 3–4 hr during and after the 2-DG infusion and no significant or consistent change was observed in either. Signs and symptoms included varying degrees of hunger, drowsiness, sweating and palpitations. In no instance were symptoms prominent enough to warrant discontinuing the test.

For comparison, an insulin tolerance test was done, using a standard dose of 0.1 U/kg body wt or more, if necessary, to induce hypoglycemia of half the fasting level or less. The insulin tolerance test was done 2–3 days before giving 2-DG to avoid depletion of adrenal medullary hormones.

A 6-hr urine collection was made to measure base-line catecholamines. Similar collections were also taken during and after the administration of insulin or infusion of 2-DG. Urinary catecholamines were measured by the method of von Euler and Floding (22) with a further modification of their fluorometric determination by the method of Price and Price (23).

Blood samples were drawn every 30 min throughout both the insulin tolerance and the 2-DG tests and changes in the glucose concentration were measured by the enzymatic method of Washko and Rice (24).

Eight normal, 8 obese, 8 acromegalic, 3 idiopathic addisonian, 4 tuberculous addisonian, 2 adrenalectomized and 1 subject with a thoracolumbar sympathectomy were studied in the clinical research ward. All tests were preceded by a 12-hr overnight fast.

Obese subjects, defined as more than 20% over ideal body wt, had no evidence of any specific endocrine disease.

Four of the acromegalic subjects, whose disease was felt to be active on the basis of clinical criteria, had elevated fasting growth hormone levels, whereas those considered inactive had normal or only slightly elevated values.

The 3 subjects classified as having idiopathic Addison's disease by clinical criteria had negative tuberculin and fungal skin tests, normal chest films and no evidence of suprarenal calcification. The four whose disease was classified as tubercular in origin had positive tuberculin and negative fungal skin tests, x-ray evidence of old calcified pulmonary complexes and, in 2 cases, of adrenal calcification. All addisonian patients were given maintenance doses of corticosteroids during the study.

Results

In Table 1 are recorded the levels of urinary epinephrine and norepinephrine output in six-hour collections taken before and after insulin hypoglycemia and cellular glucopenia induced by 2-DG in all subjects.

Although the normal, obese and acromegalic subjects had a significant increase with insulin hypoglycemia, 2-DG produced a more marked and consistent response.

At first the urine collections were taken in two successive periods of three hours each. However, because nearly all subjects still had elevated levels in the second three hours, though the highest values occurred in the first, we decided to collect for a full six hours.

Mean urinary norepinephrine output (μg/6 hr) varied considerably during the insulin tolerance test and 2-DG study compared to the basal period and no specific pattern of response was noted in any group. Since most of the norepinephrine is produced from extra-adrenal chromaffin tissue, which was intact in the subjects studied, a variable response related to the stress of the study may occur.

The two adrenalectomized subjects had no significant increase in urinary epinephrine during either insulin hypoglycemia or cellular glucopenia induced by 2-DG, compared to normal subjects. The small amount of epinephrine noted is presumably from extra-adrenal chromaffin tissue.

An intact sympathetic nervous system, especially those branches originating from the midthoracic area, is important for maintaining the functional

TABLE 1. Urinary catecholamine excretion (μg/6 hr)

Subjects	Age (yr)	Sex	Basal period		Insulin tolerance study		2-DG infusion	
			Epineph.	Norepineph.	Epineph.	Norepineph.	Epineph.	Norepineph.
Normal								
1	33	M	0.6	10.2	1.1	10.0	61.5	5.9
2	34	M	2.3	14.2	—	—	34.4	27.5
3	22	F	1.0	21.0	8.4	40.7	21.8	15.9
4	21	M	1.5	10.0	7.8	22.0	16.9	15.6
5	29	M	*	5.5	—	—	29.1	12.7
6	31	M	2.0	3.0	—	—	30.0	31.0
7	23	M	1.0	8.0	—	—	18.0	20.0
8	16	M	0.5	18.0	5.0	10.0	31.0	25.0
Mean ±SE			1.1	11.2	5.6	20.7	30.3	19.2
			±0.3	±2.2	±1.6	±7.1	±5.0	±2.9
Obesity								
1	40	F	0.9	23.5	2.0	7.6	17.2	*
2	22	F	0.5	15.7	1.9	12.2	14.4	36.1
3	14	F	0.3	5.3	3.9	12.4	42.4	14.5
4	24	F	*	15.7	3.5	10.3	28.6	8.0
5	14	F	*	6.2	1.4	8.7	9.9	4.2
6	43	M	1.0	72.0	8.0	23.0	71.0	32.0
7	24	F	0.5	9.0	—	—	36.0	45.0
8	28	M	1.0	10.0	—	—	30.9	13.3
Mean ±SE			0.5	19.7	3.5	12.4	31.3	19.1
			±0.2	±7.7	±1.1	±2.2	±6.9	±5.8
Active Acromegaly								
1	40	M	1.6	16.4	—	—	63.2	14.2
2	53	M	0.8	4.3	4.0	8.0	21.7	10.9
3	26	M	1.0	8.0	2.8	13.1	27.6	8.3
4	26	M	1.6	16.6	2.2	8.4	18.4	31.8
5	32	M	*	14.0	4.9	0.0	37.0	39.5
Inactive Acromegaly								
1	57	M	*	29.0	7.8	55.5	26.1	27.5
2	38	M	1.3	10.0	1.9	22.8	18.4	9.7
3	39	M	2.5	18.7	6.1	16.0	35.9	24.8
Mean ±SE			1.1	14.6	4.2	17.7	31.0	20.8
			±0.1	±2.7	±0.8	±6.7	±5.2	±4.3
Idiopathic Addison's Disease								
1	29	M	0.5	7.0	6.9	12.1	10.6	6.2
2	52	M	*	7.5	8.0	20.0	12.0	27.0
3	21	M	*	4.7	9.0	1.0	15.0	38.0
Mean ±SE			0.2	6.4	7.9	11.0	12.5	23.7
			±0.1	±0.8	±0.6	±5.4	±1.3	±9.1
Tuberculous Addison's Disease								
1	55	F	1.1	13.7	—	—	1.2	17.5
2	48	F	*	43.0	*	19.0	1.0	12.0
3	48	M	2.2	10.8	1.0	10.0	5.4	4.6
4	49	F	2.0	3.4	2.0	8.0	3.7	8.5
Mean ±SE			1.3	17.7	1.0	12.3	2.8	10.6
			±0.8	±13.0	±0.7	±4.1	±1.2	±3.0
Adrenalectomy								
1	23	M	3.2	*	—	—	3.0	14.0
2	50	F	1.3	21.8	1.0	45.9	1.2	48.5
Mean ±SE			2.2	10.9	1.0	45.9	2.1	31.2
			±0.2	±5.4			±0.4	±8.6
Sympathectomy								
1	59	F	0.7	4.4	*	23.4	1.6	42.4

* Not detectable by current method. Lower limit of sensitivity for epinephrine is 0.02 μg and for norepinephrine 0.05 μg/15 ml aliquot of a 24-hr collection of urine. The upper limit of normal for urinary epinephrine is 26 μg and for norepinephrine 87 μg/24 hr.

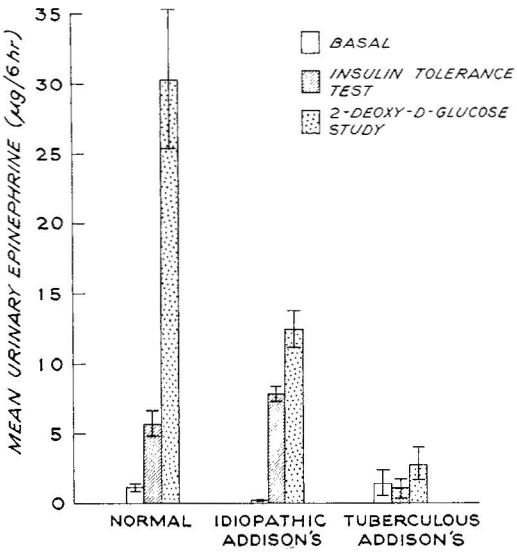

FIG. 1. Mean urinary epinephrine output after an insulin tolerance test and 2-deoxy-D-glucose infusion, compared to a basal value, is shown in normal, idiopathic addisonian and tuberculous addisonian subjects.

integrity of the adrenal medulla, as demonstrated in one subject who had had a bilateral thoracolumbar sympathectomy and whose urinary epinephrine failed to increase significantly after stimulation with 2-DG (Table 1).

The results of studies done in subjects with Addison's disease are represented in Fig. 1. Those whose disease was classified as idiopathic by clinical criteria had a significant increase in epinephrine with insulin hypoglycemia that was even more pronounced after 2-DG stimulation. Their increase with 2-DG, however, was not as marked as that seen in the normal group, suggesting that function of the adrenal medulla may be mildly impaired in these patients. The group clinically classified as having tuberculous Addison's disease failed to increase epinephrine significantly with either insulin hypoglycemia or 2-DG administration. The subject with the highest epinephrine output in the tuberculous group (Table 1, Subject 3) had prominent bilateral adrenal calcification as well as bilateral parenchymal chest lesions, but also had more sweating than usual upon administration of 2-DG, suggesting that a moderate peripheral sympathetic nervous system response occurred with this stimulus.

Blood glucose levels during administration of 2-DG are listed in Table 2 for each subject. These levels corresponded closely to the urinary epinephrine response (Fig. 2). In the subjects with intact adrenal glands, in whom the urinary epinephrine output was highest, the greatest increase in blood glucose was noted. The adrenalectomized, sympathectomized and tuberculous addisonian subjects who had the least increase in urinary epinephrine similarly had only a minimal increase in blood glucose. The group with idiopathic Addison's disease, which had an inter-

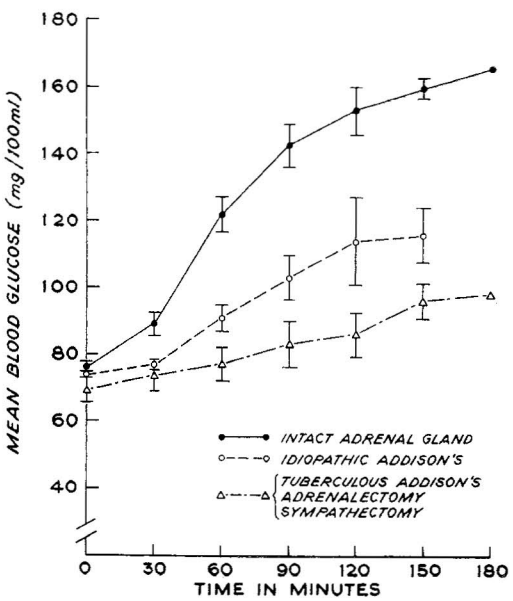

FIG. 2. Mean blood glucose values obtained during and after 2-deoxy-D-glucose infusion are shown in subjects with intact adrenal glands (normal, obese and acromegalic) and in subjects with impairment of adrenal function.

TABLE 2. Blood glucose levels during the 2-deoxy-D-glucose infusions

Subjects	Blood glucose (mg/100 ml)						
	0 min	30 min	60 min	90 min	120 min	150 min	180 min
Normal							
1	69	85	150	176	176	190	188
2	82	90	118	137	148	162	—
3	73	108	148	160	179	186	190
4	76	82	98	106	108	109	108
5	78	78	104	125	134	136	142
6	78	88	134	158	176	188	—
7	75	128	169	180	192	204	—
8	86	92	133	145	152	150	—
Obesity							
1	80	90	116	133	142	—	—
2	70	75	88	108	120	131	—
3	69	84	150	208	214	234	231
4	70	75	104	138	148	152	—
5	76	84	116	131	140	138	148
6	74	92	144	187	214	222	239
7	82	84	117	147	172	174	176
8	79	126	149	162	160	166	—
Active Acromegaly							
1	78	86	142	170	180	188	189
2	72	72	98	123	131	140	142
3	86	95	97	108	108	112	112
4	86	88	108	124	127	128	131
5	87	92	114	133	140	139	—
Inactive Acromegaly							
1	74	81	98	109	116	110	—
2	70	79	94	104	114	114	—
3	66	97	154	179	202	218	—
Mean ±SE of Subjects with Intact Adrenal Glands							
	76.5 ±1.3	89.6 ±2.9	122.6 ±4.9	143.8 ±6.0	153.4 ±6.6	160.5 ±3.27	166.3 ±42.8
Idiopathic Addison's Disease							
1	74	77	86	99	101	108	—
2	74	78	98	115	141	134	—
3	74	78	91	95	100	107	—
Mean ±SE of Subjects with Idiopathic Addison's Disease							
	74.0 ±0.0	77.6 ±0.3	91.6 ±3.5	103.0 ±6.1	114.0 ±13.6	116.3 ±8.8	
Tuberculous Addison's Disease							
1	88	92	100	112	114	109	—
2	66	68	69	76	78	80	—
3	67	76	82	92	94	102	—
4	68	82	82	86	91	93	—
Adrenalectomy							
1	55	56	62	66	67	—	—
2	64	67	71	73	76	—	—
Sympathectomy							
1	78	80	75	79	84	99	98
Mean ±SE of Subjects with Diminished Adrenomedullary Reserve							
	69.4 ±4.3	74.4 ±4.7	77.3 ±4.9	83.4 ±6.1	86.3 ±6.1	96.6 ±5.0	98.0

mediate epinephrine response, had a response in blood glucose intermediate between the other two groups.

Discussion

In the study of the adrenal medulla and its physiology, it has been noted that basal values for epinephrine and norepinephrine are often low in normal subjects. To evaluate its functional capacity in man various means of stimulation have been used, which include insulin (2–5, 7, 8, 11, 25–31), methacholine (25), lysergic acid diethylamide (32), nicotine (33–35), 2-DG (12, 13) and various forms of physical and mental stress (36, 37).

Since the initial work by von Euler and Luft (11) in man, insulin hypoglycemia has been the stimulus most effectively and frequently used to increase the output of epinephrine in man. Luft and von Euler (28), using a biologic assay, noted only minimal increases in urinary epinephrine after insulin hypoglycemia in five addisonian and one adrenalectomized subject, and Brunjes, Hodgman, Nowack and Johns (38) did not observe any significant rise in the addisonian subjects they studied. Later, using a fluorometric assay for epinephrine, von Euler, Ikkos and Luft (31) were able to demonstrate a rise in epinephrine output in adrenalectomized subjects, though much less marked than in their normal subjects. They concluded that extra-adrenal chromaffin cells produce epinephrine in response to insulin hypoglycemia. An increase in epinephrine output has been noted after administration of 2-DG in the rat (15) and in man (12, 13).

2-DG is phosphorylated in the cell to 2-DG-6-phosphate and acts as an inhibitor of glucose metabolism in the cell by inhibition of the enzyme phosphoglucoisomerase (39, 40). Nirenberg and Hogg (39) found that 2-DG-6-phosphate also inhibited fructose-6-phosphate and fructose-1,6-diphosphate in homogenates, suggesting inhibition of a further step in the Embden-Meyerhof pathway. These sites of inhibition create a state of cellular glucopenia which can then act to stimulate other physiologic processes. In our studies, the subjects with intact adrenal glands (normal, obese, acromegalic) were found to have a marked increase in urinary output of epinephrine (mean 30-fold) compared to those who were adrenalectomized, sympathectomized, or in whom the adrenal medulla had been destroyed by tuberculosis. In the one subject in our group who had a bilateral thoracolumbar sympathectomy, epinephrine increased only slightly upon administration of 2-DG. This suggests that 2-DG does not affect the adrenal medulla directly.

In animals, adrenal denervation decreases epinephrine output (15) and a decreased hyperglycemic response to 2-DG administration was noted after administration of an adrenergic blocking agent (dihydroergotamine) (18, 21). Experimental studies by Hökfelt (41), Vogt (42) and Duner (43) demonstrated that secretory activity of the adrenal medulla, although it is decreased, can continue despite denervation. The importance of an intact sympathetic nervous system is demonstrated by our case and by recent experimental findings by Cantu, Wise, Goldfien, Gullixson, Fischer and Ganong (44) which have shown the midthoracic portion of the spinal cord to be essential for the adrenal medullary response to hypoglycemia.

Administration of 2-DG in varying doses has been shown to significantly increase the blood glucose in experimental animals with intact adrenal glands

(15–18, 20, 21) but does so to a lesser extent in adrenalectomized ones (17–20).

Among our subjects, those with intact adrenal glands had a pronounced increase in epinephrine output and hyperglycemia upon infusion with 2-DG. The patients who were adrenalectomized, sympathectomized, or had clinical evidence of tuberculous Addison's disease did not show a significant rise in epinephrine or glucose. The subjects with idiopathic Addison's disease, who had an intermediate response of epinephrine to 2-DG, also had a pattern of glucose response intermediate between those with normal glands and those who had no response. We were able to estimate the subjects' epinephrine output by their blood glucose response. This study demonstrates a close correspondence between the urinary epinephrine output and the blood glucose level, suggesting that epinephrine is the primary factor in hyperglycemia occurring after 2-DG administration.

Using insulin hypoglycemia as the stimulus and a biologic assay to measure the catecholamines, von Euler and Luft (11) were able to find a significant increase in epinephrine in only one of six cases of acromegaly. Pathology reports in patients with acromegaly have usually described their adrenal chromaffin tissue as normal, although hyperplasia (45) and occasional "macroscopic absence of the adrenal medulla" have been described (46). The acromegalic subjects studied in our series had a pronounced response to 2-DG stimulation in their epinephrine levels, suggesting that their medullas were intact and functioning.

In the extensive review of Addison's disease by Guttman in 1930 (47) it was noted that the incidence of tuberculosis as its cause was about 70%. By 1948 tuberculosis had decreased in frequency and Friedman (48) found that primary atrophy was the cause in 60% and tuberculosis in 40% of cases.

In Addison's disease due to tuberculosis the adrenal medulla is involved and destroyed. By injecting virulent tuberculosis bacteria into the adrenal glands of dogs, De Vecchi (49) demonstrated that the medulla was first completely destroyed and then the process slowly involved the cortex. Evidence that a similar sequence of pathologic change occurs in man has been seen in necropsy studies, in which the medulla is usually noted to be destroyed, whereas portions of the cortex may remain (47, 50).

In most cases of idiopathic Addison's disease the medulla is described as normal or only slightly atrophied (47, 48, 50–53). Infiltration of small round cells is occasionally noted (47, 48, 54).

Recent studies (55, 56) have shown that some patients with Addison's disease have antibodies against adrenal tissue, suggesting an autoimmune mechanism of adrenocortical destruction in these cases. Our three subjects with idiopathic Addison's disease were found to have antibodies to adrenal tissue, whereas three of four of our tuberculous addisonians had no evidence of adrenal antibodies.[1]

The subjects in this study who were classified as having idiopathic Addison's disease because they had no evidence of tuberculosis, fungal infection or other systemic illness had a consistent and significant (mean 12-fold) increase in epinephrine output upon stimulation with 2-DG. This response was less marked than in the group with clinically intact adrenal glands, suggesting a minor degree of functional impairment.

The fungi are rarely reported as caus-

[1] We are indebted to Dr. K. Wuepper for performing the adrenal antibody studies.

ative organisms for Addison's disease. Destruction of the adrenal medulla has been noted in histoplasmosis (52, 57, 58) and coccidioidomycosis (59). In most cases of amyloidosis of the adrenal causing Addison's disease, the gland has been described as normal, although encroachment on (52), minor involvement (60, 61) and even complete destruction (62) of the adrenal medulla have occasionally been noted in a few. Thrombosis of the adrenal veins producing Addison's disease may also give rise to medullary destruction (47). Thus, although other pathologic processes causing Addison's disease can destroy the adrenal medulla, they are quite rare and can usually be recognized by clinical evaluation, skin tests, or other investigative procedures.

In most patients infected with tuberculosis there is no clinical evidence of Addison's disease or pathologic change in the adrenal gland (47). Because of the relatively high incidence of positive tuberculin skin tests and low incidence of Addison's disease in the general population, a positive test does not necessarily establish the Addison's disease as tuberculous. Because the adrenal medulla is usually destroyed in the tuberculous form of the disease but is usually intact in the idiopathic type, a test designed to stimulate the medulla selectively should allow the distinction to be made with relative certainty. Close supervision of the tuberculous addisonian to discover possible reactivation of his disease process elsewhere is important.

Although adrenal medullary damage has not been confirmed by pathologic studies in our cases, it would appear that infusion of 2-DG is capable of increasing output of epinephrine by the adrenal medulla to a significant degree. Its usefulness in differentiating idiopathic and tuberculous Addison's disease should be evaluated further. However, these preliminary data suggest that it may be of clinical value in making this distinction. It should also be of value in evaluating other disorders in which the function of the adrenal medulla is important to the understanding of their pathophysiology.

Acknowledgments

The idea for this work stems from a conversation that one of us (PHF) had with Professor José Garcia-Reyes during a visit in Mexico City. It was he who mentioned his early attempts to differentiate between the 2 types of Addison's disease, *viz.*, tuberculous *vs.* atrophic adrenal cortical states, by investigating the output of epinephrine under insulin or reserpine stimulation. To us, 2-DG appeared to be a more effective substance to work with and we set out to do so in a study based on Professor Garcia-Reyes' original idea. We wish to thank Dr. John H. Karam for continued help in the management of patients, and for performing some of the insulin tolerance tets.

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