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EFFECT OF REPEATED ADMINISTRATION OF 2-DEOXY-D-GLUCOSE OR INSULIN ON CATECHOLAMINE-SYNTHESIZING ENZYMES IN RAT ADRENALS

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Abstract—One dose of either 2-deoxy-D-glucose (2DG) or insulin does not alter significantly enzymes involved in the synthesis of adrenal catecholamines. However, repeated doses of 2DG (500 mg/kg) or insulin (10 units of Iletin/kg) injected daily into male rats produce striking changes in levels of these enzymes. Six hr after the last of seven daily injections of either agent, there was significant increase of adrenal weight and marked rise of adrenal tyrosine hydroxylase and dopamine-B-hydroxylase. Adrenal phenylethanolamine-*N*-methyl transferase was increased only slightly by single or repeated doses of 2DG or insulin but attained statistical significance only after repeated doses of insulin. 2DG apparently was a greater stimulus for epinephrine secretion than insulin since a marked lowering of adrenal epinephrine was induced by 2DG but not by insulin.

EXCRETION of urinary epinephrine after intravenous infusion of 2-deoxy-D-glucose (2DG), an inhibitor of glucose metabolism, has been used to evaluate adrenomedullary reserve in the rat and in man.[1] The drug causes inhibition of glucose utilization, relative intracellular hypoglycemia and a rise in both plasma glucose and free fatty acids,[2,3] presumably as a consequence of release of epinephrine (E) from the adrenal medulla. The latter is reflected by a consistent and pronounced increase in urinary epinephrine.[4] In rats, insulin treatment results in an increase in adrenal tyrosine hydroxylase (TH) and a transient decrease in dopamine-B-hydroxylase (DBH).[5] In rabbits after a single insulin treatment, TH levels rise to above normal by 48 hr and reach twice normal at 96 hr.[6] DBH activity decreases at 3 hr, then increases but does not exceed normal by more than 40 per cent. Although 2DG has been favored over insulin as an agent for evaluation of adrenomedullary reserve,[1] the effects of repeated administrations of 2DG on adrenal tyrosine hydroxylase, dopamine-B-hydroxylase and phenylethanolamine-*N*-methyl transferase (PNMT) have not been reported. The present investigation examines the effects of successive daily doses of 2DG or insulin on the activity of these adrenomedullary enzymes in rat adrenal glands.

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METHODS

Groups of six to ten male Sprague–Dawley rats weighing 180–250 g were injected subcutaneously for 1 or 7 days with 2DG (500 mg/kg) or with two units regular insulin (Iletin). Control animals received injections of media. All the rats had water and food *ad lib.*

Six hr after administration of the last dose of either drug, the rats were decapitated and adrenal glands were removed, cleaned, weighed and homogenized in 1·0 ml ice-cold isotonic sucrose. An aliquot (100 μl of the sucrose homogenate) was added to 0·9 ml 0·4 N perchloric acid for assay of catecholamines.[7] An aliquot of remaining homogenate was centrifuged at 26,000 *g* for 20 min and aliquots of the clear supernatant fluid assayed for TH using the method of Nagatsu *et al.*[8] and for PNMT using the technique of Axelrod.[9] Another aliquot (100 μl) was added to 200 μl 0·15% Triton X-100. The mixture was refrigerated overnight, and was assayed for DBH using the procedure of Friedman and Kaufman[10] as modified by Viveros *et al.*[11]

Two groups of Sprague–Dawley rats were placed in individual metabolic cages 1 week before experiment started and fed *ad lib.* After collection of urine for 24 hr, one group received 500 mg/kg body weight of 2DG subcutaneously while the other group received only water; and urine was collected for a second day. Urines were analyzed for epinephrine and norepinephrine.[7]

RESULTS

Effect of 2DG or insulin administration on adrenal weights and catecholamine levels

Six hr after a single injection of either 2DG or insulin there was no alteration in the weight of the adrenal glands. When injected daily for 1 week, both 2DG and insulin produced significant increases in adrenal weights. The animals given 2DG seven times

TABLE 1. EFFECTS OF 2-DEOXY-D-GLUCOSE (2DG) OR INSULIN ADMINISTRATION ON ADRENAL WEIGHTS AND CATECHOLAMINE LEVELS IN RATS*

Group	Adrenal weight (mg/pair)	Epinephrine (μg/pair)	Norepinephrine (μg/pair)
Control	39·3 ± 0·8	23·33 ± 1·91	6·01 ± 0·46
1 × 2DG	41·8 ± 1·0	12·01 ± 0·54§	4·55 ± 0·46†
7 × 2DG	50·2 ± 1·7§	6·79 ± 0·43§	4·67 ± 0·32†
1 × Insulin	42·6 ± 2·2	21·73 ± 1·21	6·48 ± 0·88
7 × Insulin	45·4 ± 1·4‡	23·62 ± 0·71	6·62 ± 0·67

* Results are expressed as mean values (± S.E.M.) per pair of adrenal glands for groups of seven or nine animals.
† Compared to control group, $P < 0·05$.
‡ Compared to control group, $P < 0·01$.
§ Compared to control group, $P < 0·001$.

showed the maximum increase in adrenal weight (Table 1), presumably as a consequence of adrenocortical hypertrophy.

A single dose of 2DG decreased epinephrine content of the adrenal to about one-half the control level. When 2DG administration was repeated for 7 consecutive days there was a further lowering of adrenal epinephrine to about one-fourth the control

level. In contrast, insulin had no significant effect on the adrenal epinephrine content whether given 1 or 7 days. There were much less striking decreases in norepinephrine levels after one or seven doses of 2DG. This, presumably, reflects a fall in total catecholamine level.

Effect of 2DG or insulin administration on levels of adrenomedullary enzymes

After a single injection, neither 2DG or insulin produced significant changes in levels of tyrosine hydroxylase (Fig. 1). However, when given daily for a week, either

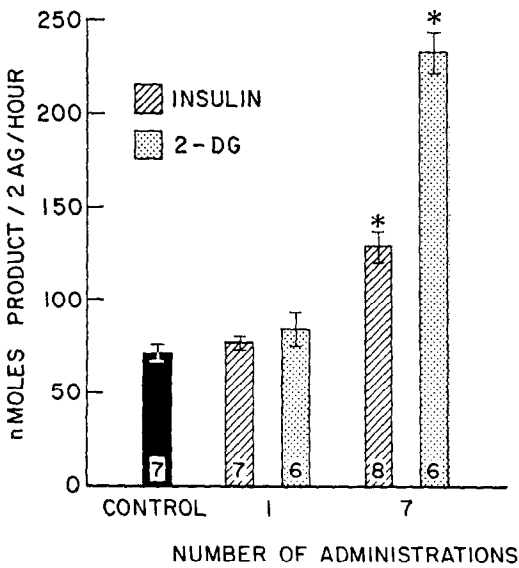

FIG. 1. Effect of 2-deoxy-D-glucose (2DG) or insulin administration on levels of adrenal tyrosine hydroxylase in rats. The number of subcutaneous administrations (once/day) of either 2DG (500 mg/kg) or regular insulin (2 units Iletin) is indicated on the abscissa. Results are expressed as n-moles product (dopa) per pair of adrenal glands and are mean values (± S.E.M.) for the number of rats indicated in the columns. * = $P < 0.001$ compared to control.

2DG or insulin significantly increased adrenal TH levels. The levels found after seven administrations of 2DG were more than three times that of controls whereas after seven injections of insulin, the values were only about twice the control (Fig. 1).

Alterations in dopamine-B-hydroxylase levels were somewhat analogous to those of tyrosine hydroxylase. Six hr after one administration of either 2DG or insulin, levels of DBH were not altered significantly (Fig. 2). Daily administration of 2DG for 1 week caused a marked rise in DBH. Insulin injected daily for 1 week produced a smaller but significant ($P < 0.01$) increase in levels of this enzyme (Fig. 2).

Levels of adrenal phenylethanolamine-*N*-methyl transferase were only slightly elevated and did not reach statistical significance except after repeated insulin injection (Table 2).

Effect of 2DG on excretion of urinary catecholamines

Urinary excretion of epinephrine was increased approximately 12-fold on the day 2DG was injected, but there was no significant change in norepinephrine excretion (Table 3).

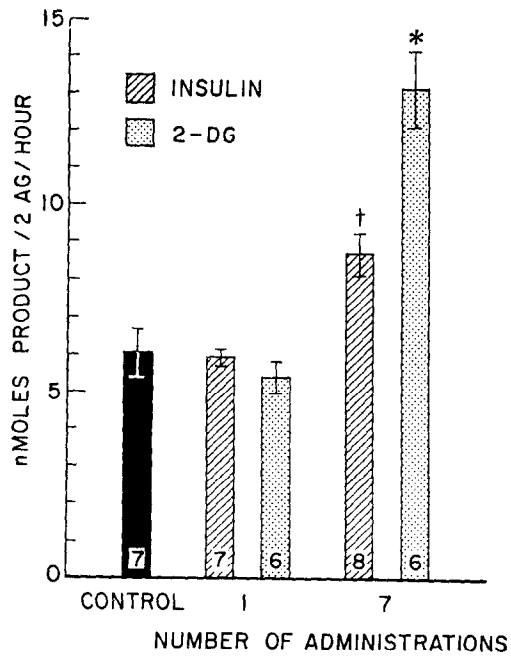

FIG. 2. Effect of 2-deoxy-D-glucose (2DG) or insulin administration on levels of dopamine-B-hydroxylase in the adrenals of rats. The number of subcutaneous administrations (once/day) of either 2DG (500 mg/kg) or regular insulin (2 units Iletin) is indicated on the abscissa. Results are expressed as nmoles product (octopamine) per pair of adrenal glands and are mean values (± S.E.M.) for the number of rats indicated in the columns. + = $P < 0.01$; * = $P < 0.001$ compared to control.

TABLE 2. EFFECT OF 2-DEOXY-D-GLUCOSE (2DG) OR INSULIN ADMINISTRATION ON LEVELS OF ADRENAL PHENYLETHANOLAMINE-*N*-METHYL TRANSFERASE (PNMT) IN RATS

Treatment*	PNMT†
Control	35·8 ± 1·4
1 × Insulin	39·7 ± 1·9
7 × Insulin	42·8 ± 1·1‡
1 × 2DG	40·8 ± 2·2
7 × 2DG	38·8 ± 1·6

* Controls received injections of 1·0 ml H_2O subcutaneously. The 1 × Insulin group received injections of 2 units of regular insulin (Iletin) subcutaneously for 1 day only. The 7 × Insulin group received the same for 7 days. The 1 × 2DG group received subcutaneously 2DG (500 mg/kg) dissolved in 1·0 ml distilled H_2O for 1 day only. The 7 × 2DG group received the same for 7 days.

† Results are expressed as units per pair of adrenal glands and are mean values (± S.E.M.) for groups of six to nine animals. One unit of enzyme activity is equivalent to one nmole product formed per hour.

‡ Compared to controls, $P < 0.01$.

TABLE 3. EFFECT OF 2-DEOXY-D-GLUCOSE (2DG) ON URINARY EXCRETION OF CATECHOLAMINES IN RATS*

Treatment†	Epinephrine (μg/24 hr)	Norepinephrine (μg/24 hr)
Control	0·27 ± 0·05	1·40 ± 0·18
2DG	3·26 ± 0·46‡	1·92 ± 0·16

* Results are expressed as μg epinephrine or norepinephrine excreted per day per animal and are mean values (± S.E.M.) for groups of five to six rats.

† Control animals received only food and water *ad lib.* Rats administered 1·0 ml H_2O subcutaneously 1 × do not differ from above control animals. The treated group received one injection of 2DG (500 mg/kg) subcutaneously.

‡ Compared to controls, $P < 0·001$.

DISCUSSION

Successive (seven) daily subcutaneous injections of either 2DG or insulin significantly elevated the weights of the adrenal gland. The adrenal cortex accounts for about 80 per cent of the adrenal weight and the increase produced by 2DG and insulin suggests that these agents produce a stress which results in some adrenocortical hypertrophy.

Injection of 2DG reduces levels of adrenal epinephrine and elevates urinary excretion of this catecholamine.[12,13] Apparently, secretion of adrenal epinephrine following 2DG is sufficiently intense to exceed the capacity for replacement of the catecholamine by synthesis. In the present study, although adrenal norepinephrine decreased after one dose of 2DG, the increased urinary excretion of this catecholamine did not attain statistical significance. It is apparent that 2DG significantly lowers adrenal levels of epinephrine and norepinephrine whether given one or seven times while insulin induces no alterations. Thus, 2DG, under the experimental conditions of this study, appears much more potent than insulin in releasing catecholamines from the adrenal medulla. It has been previously demonstrated that insulin can diminish levels of adrenal catecholamines.[1,14] Adrenal catecholamine response to insulin may vary with strain of rat, dose, dietary condition and season.[14] To achieve the maximal effect of insulin for a given dosage, one would expect that rats should be fasted rather than fed *ad lib.* as in this study. After adrenal denervation by transection of the spinal cord or transection of the splanchnic nerves, 2DG does not cause change in adrenal content of catecholamines.[12] Thus, the effect of 2DG on release of catecholamines appears mediated by impulses originating in the central nervous system.

After adrenal epinephrine depletion with repeated immobilization,[15] there is an increased rate of catecholamine resynthesis with attending increased levels in catecholamine-synthesizing enzymes.[16,17] These enzymes are also increased after 2DG- or insulin-induced catecholamine release. The increases in TH and DBH found by Kirshner *et al.*[5,6] 96 hr after a single dose of insulin are of the same magnitude as those found after repeated doses of insulin in the present study. However, both TH and DBH increase more after repeated 2DG than after repeated insulin administration (Figs. 1 and 2). While all treatments appear to increase PNMT slightly, a statistically significant increase was found only after repeated administration of insulin. Repeated

immobilization stress and repeated 2DG administration both similarly increase TH[16] and DBH[17] levels about 3-fold (Figs. 1 and 2). Maximal elevation of TH activity appears to be about 300–400 per cent above control levels since this magnitude of increase was observed after 2DG injection (Table 1), after immobilization,[16] and following reserpine or 6-hydroxydopamine administration.[18] The increase in activity of adrenal TH and DBH is dependent mostly on intact adrenal innervation.[16,17,19] Thus, the effect of 2DG on TH and DBH is mainly through splanchnic nerve stimulation. This is consistent with the observation that after splanchnic nerve section 2DG does not decrease adrenal epinephrine levels.[12]

Although TH is believed to be rate-limiting in catecholamine synthesis,[20] increases in TH levels are associated with increases in DBH levels. The reason for this is not clear. DBH is released from the adrenal along with catecholamine[11] and the synthesis of the enzyme is accelerated when such release occurs.[17] The ratio of DBH to catecholamines in adrenal perfusate is similar to that of the soluble content of the vesicles from which they are released.[11] If only part of the newly synthesized DBH is available for release, accelerated synthesis might lead to increased levels of the enzyme.

Adrenal PNMT activity is regulated mostly by the pituitary–adrenocortical system.[16,19,21] In the present study, although 2DG administration caused increases in adrenal weight, it did not produce significant changes in PNMT levels. Possibly adrenal steroidogenesis was not sufficiently increased by the injection of 2DG to significantly increase PNMT activity. It is apparent, however, that the elevated excretion of epinephrine after administration of 2DG is attended by marked increases in adrenal TH and DBH, but not PNMT. Hence it appears unlikely that PNMT limits the rate of epinephrine production by the adrenal. Thus, in addition to showing that 2DG is a more effective agent than insulin for evaluating adrenal medullary reserve, the results of this study suggest that PNMT is not rate-limiting in epinephrine synthesis.

REFERENCES

1. L. C. WEGIENKA, S. G. GRASSO and P. H. FORSHAM, *J. Clin. Endocr. Metab.* **26,** 37 (1966).
2. J. LAZLO, W. R. HARLAN, R. F. KLEIN, N. KIRSHNER, E. H. ESTES, JR. and M. D. BOGDANOFF, *J. clin. Invest.* **40,** 171 (1961).
3. W. R. HARLAN, J. LAZLO, M. D. BOGDANOFF and E. H. ESTES, JR., *J. clin. Endocr. Metab.* **23,** 41 (1963).
4. S. G. GRASSO, J. H. KARAM, L. C. WEGIENKA, G. M. GRODSKY and P. H. FORSHAM, *J. clin. Endocr. Metab.* **28,** 535 (1968).
5. R. L. PATRICK and N. KIRSHNER, *Fedn Proc. Fed. Am. Soc. Exp. Biol.* **29,** 277 (1970).
6. O. H. VIVEROS, L. ARQUEROS, R. J. CONNETT and N. KIRSHNER, *Molec. Pharmac.* **5,** 69 (1969).
7. A. H. ANTON and E. D. SAYRE, *J. Pharmac. exp. Ther.* **138,** 360 (1962).
8. T. NAGATSU, M. LEVITT and S. UDENFRIEND, *Analyt. Biochem.* **9,** 122 (1964).
9. J. AXELROD, *J. biol. Chem.* **237,** 1657 (1962).
10. S. FRIEDMAN and S. J. KAUFMAN, *J. biol. Chem.* **240,** 4763 (1965).
11. O. H. VIVEROS, L. ARQUEROS and N. KIRSHNER, *Life Sci.* **7,** 609 (1968).
12. B. HOKFELT and S. BYGDEMAN, *Proc. Soc. exp. Biol. Med.* **106,** 537 (1961).
13. D. G. JOHNSON, *Acta physiol. scand.* **65,** 337 (1965).
14. B. HOKFELT, *Acta physiol. scand. Suppl.* **92,** 25 (1951).
15. R. KVETŇANSKÝ and L. MIKULAJ, *Endocrinology* **87,** 738 (1970).
16. R. KVETŇANSKÝ, V. K. WEISE and I. J. KOPIN, *Endocrinology* **87,** 744 (1970).
17. R. KVETŇANSKÝ, V. K. WEISE, G. GEWIRTZ and I. J. KOPIN, *Molec. Pharmac.* **7,** 81 (1971).
18. H. THOENEN, R. A. MUELLER and J. AXELROD, *Nature, Lond.* **221,** 1264 (1969).
19. R. KVETŇANSKÝ, G. GEWIRTZ, V. K. WEISE and I. J. KOPIN, *Endocrinology* **87,** 1323 (1970).
20. M. LEVITT, S. SPECTOR, A. SJOERDSMA and S. UDENFRIEND, *J. Pharmacol. exp. Ther.* **148,** 1 (1965).
21. R. J. WURTMAN and J. AXELROD, *J. biol. Chem.* **241,** 2301 (1966).