Elevation of Blood Glucose and Induction of Hepatic Enzymes by 2-Deoxyglucose in Mice with Hypothalamic Damage Caused by Gold Thioglucose

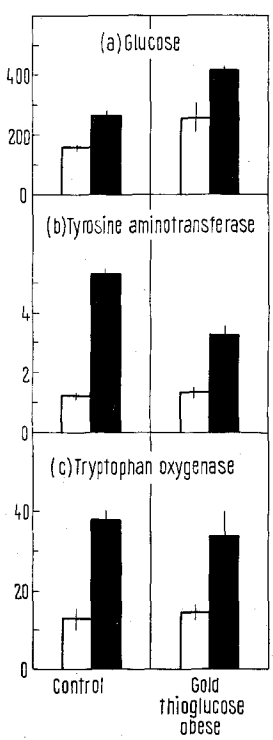
These experiments were done to test a possible involvement of the ventromedial hypothalamus in the induction of two hepatic enzymes, tyrosine aminotransferase and tryptophan oxygenase, by 2-deoxyglucose. Both enzymes turn over rapidly and are increased by various extrahepatic stimuli. For instance, the enzymes vary in a daily rhythm in mice and rats with free access to food[1–5]; the rhythm in the enzymes is dependent upon rhythmic food intake in such animals and can be altered by changing the feeding rhythm[6,7]. We have previously shown that mice without an intact ventromedial hypothalamus, i.e. mice made obese by treatment with gold thioglucose (GTG), do not exhibit the normal daily rhythm in hepatic tyrosine aminotransferase and do not increase their hepatic tyrosine aminotransferase in response to a 48 h fast[4,7]. Based on these experiments, we have considered that the hypothalamus may be involved in the regulation of tyrosine aminotransferase (neural regulation of that enzyme has also been proposed by BLACK and AXELROD[8]) and of tryptophan oxygenase (for which hypothalamic regulation has been demonstrated by SHIMAZU[9]).

The daily rhythmic change of hepatic tyrosine aminotransferase and tryptophan oxygenase resembles the rhythm in circulating free fatty acids and is opposite to the rhythm of hepatic glycogen[10]. GOLDFIEN[11] has suggested that 'mechanisms for the storage and retrieval of substrates for intermediary metabolism are required for the intermittent feeding pattern observed in many species. The integrative function of the central nervous system and its unique dependence on glucose for energy metabolism suggest that it might play an important role in the regulation of these mechanisms'. The rhythmic changes in the amino acid-metabolizing enzymes and the glycogen content of liver and of circulating free fatty acids in plasma may be mechanisms like those GOLDFIEN described. If so, the hypothalamus might be expected to have some direct or indirect role in their regulation.

GOLDFIEN et al.[12] published evidence that the mobilization of plasma free fatty acids by 2-deoxyglucose involves neural pathways. DESIRAJU et al.[13] reported altered firing of single neurons in the hypothalamic feeding centers after 2-deoxyglucose administration. HOKFELD and BYDGEMAN[14] had earlier suggested that 2-deoxyglucose acted on a centrally located receptor to increase epinephrine secretion from the adrenal medulla and in turn elevate blood glucose. LIKUSKI et al.[15] have suggested that 2-deoxyglucose acts on the same cells in the satiety center of the hypothalamus that are destroyed by GTG. RIDLEY and CIRPILI[16] reported that the stimulation of gastric secretion induced by 2-deoxyglucose did not occur in rats with hypothalamic lesions. These observations suggest that at least some of the metabolic actions of 2-deoxyglucose are secondary to its effect on hypothalamic neurons. The idea that hypothalamic centers that control feeding behavior also influence endocrine regulation of metabolism has previously received attention[17,18].

2-Deoxyglucose has been shown to induce tyrosine aminotransferase in rats[19]. The possibility that the failure of GTG-lesioned mice to show a daily rhythm in hepatic tyrosine aminotransferase and an increase of that enzyme during starvation is due to a loss of hypothalamic function that would be required for an effect by 2-deoxyglucose led us to determine if tyrosine aminotransferase and tryptophan oxygenase in these mice were affected by 2-deoxyglucose.

Male albino mice were obtained from a local supplier and had free access to standard laboratory chow and water. At the time part of the mice were treated with GTG (800 mg/kg, i.p.), their body weights ranged between 16 and 21 g. 4 months later, when this experiment was



Effects of 2-deoxyglucose (solid bars) in control and gold thioglucose obese mice compared to saline treatment (open bars). a) Plasma glucose in mg/100 ml; b) hepatic tyrosine aminotransferase in μmoles *p*-hydroxyphenylpyruvate formed per min/g (wet weight) of tissue; c) hepatic tryptophan oxygenase in nmoles kynurenine formed per min/g (wet weight) of tissue. Means and standard errors for 5 mice per group are shown.

[1] R. J. WURTMAN and J. AXELROD, Proc. natn. Acad. Sci., USA *57*, 1594 (1967).
[2] M. CIVEN, R. ULRICH, B. M. TRIMMER and C. B. BROWN, Science *157*, 1563 (1967).
[3] G. E. SHAMBAUGH, D. A. WARNER and W. R. BEISEL, Endocrinology *81*, 811 (1967).
[4] R. W. FULLER and I. H. SLATER, Fedn. Proc. *27*, 403 (1968).
[5] M. I. RAPOPORT, R. D. FEIGIN, J. BRUTON and W. R. BEISEL, Science *153*, 1642 (1966).
[6] R. W. FULLER and H. D. SNODDY, Science *159*, 738 (1968).
[7] R. W. FULLER, in *Enzyme Synthesis and Degradation in Mammalian Systems* (Ed.: M. RECHCIGL, Karger, Basel 1971).
[8] I. B. BLACK and J. AXELROD, Fedn. Proc. *28*, 729 (1969).
[9] T. SHIMAZU, Biochim. biophys. Acta *65*, 373 (1962).
[10] R. W. FULLER and E. R. DILLER, Metabolism *19*, 226 (1970).
[11] A. GOLDFIEN, Pharmac. Rev. *18*, 303 (1966).
[12] A. GOLDFIEN, K. S. GULLIXSON and G. HARGROVE, J. Lipid Res. *7*, 357 (1966).
[13] T. DESIRAJU, M. G. BANERJEE and B. K. ANAND, Physiol. Behav. *3*, 757 (1968).
[14] B. HOKFELT and S. BYDGEMAN, Proc. Soc. exp. Biol. Med. *106*, 537 (1961).
[15] H. J. LIKUSKI, A. F. DEBONS and R. J. GLOUTIER, Am. J. Physiol. *212*, 669 (1967).
[16] P. RIDLEY and E. CIRPILI, Am. J. dig. Dis. *14*, 262 (1969).
[17] D. A. BOOTH, E. E. COONS and N. E. MILLER, Physiol. Behav. *4*, 991 (1969).
[18] D. A. BOOTH and N. E. MILLER, Physiol. Behav. *4*, 1003 (1969).
[19] N. FRIEDMAN and H. E. WERTHEIMER, Metabolism *15*, 230 (1966).

performed, the weight in grams of the 10 control mice was 39.8 ± 1.4 SEM The GTG-treated mice averaged 60.7 ± 2.0 g. The difference in weight was highly significant ($P < 0.001$). All of the GTG-treated mice differed from the control mean weight by more than 2 standard deviations (which was our criterion for ensuring that hypothalamic damage had resulted from the GTG).

2-Deoxy-D-glucose (500 mg/kg, s.c.) or saline was injected at noon into 5 mice per group. 2 h later, the mice were killed by decapitation. Blood was collected into heparinized tubes; plasma was prepared and analyzed for glucose by a glucose oxidase method. The livers were rapidly removed and frozen on dry ice. Later they were homogenized, and the $100{,}000 \times g$ supernatant fraction was used for enzyme assays[20,21].

The Figure a shows plasma glucose levels. The GTG-lesioned mice in this experiment were hyperglycemic. The percentage increase in blood glucose concentration caused by 2-deoxyglucose was about equal in control and in GTG-treated mice. Basal activity of hepatic tyrosine aminotransferase (Figure b) in GTG-lesioned mice was approximately equal to that in intact mice. 2-Deoxyglucose significantly increased the enzyme in both groups, although the response in the GTG-lesioned mice was only about half that in the controls. YUWILER et al.[22] have shown that glucose feeding antagonized the induction of tyrosine aminotransferase by glucocorticoids, tryptophan, and casein. Possibly the high blood glucose level in the GTG mice treated with 2-deoxyglucose suppressed the induction of tyrosine aminotransferase. But YUWILER et al. also found that glucose antagonized tryptophan oxygenase induction, and tryptophan oxygenase induction by 2-deoxyglucose was as great in the GTG mice as in controls (Figure c). Indeed, hepatic tryptophan oxygenase activity was almost identical in GTG-treated and in control mice both before and after treatment with 2-deoxyglucose. These experiments show that 2-deoxyglucose induces hepatic tyrosine aminotransferase and tryptophan oxygenase both in intact mice and in mice with GTG-induced hypothalamic lesions. An intact ventromedial hypothalamus is thus not required for these effects of 2-deoxyglucose.

The mechanism by which 2-deoxyglucose induces these enzymes is not established. We have earlier shown that tyrosine aminotransferase is induced by epinephrine[23], so that stimulation of epinephrine secretion from the adrenal medulla is a possible mechanism. That stimulation of epinephrine secretion in fact occurred after 2-deoxyglucose administration to both groups of mice is suggested by the similar elevation in blood glucose levels. Plasma free fatty acid levels measured at the time of sacrifice of the mice in our study were not elevated in the 2-deoxyglucose-treated group, but that is no doubt because the time interval of 2 h after drug treatment was too long; RICHARDSON and HOKFELT[24] found maximum increase in plasma FFA $^1/_2$ h after 2-deoxyglucose treatment with little effect remaining at 2 h.

In summary, 2-deoxyglucose increased hepatic tyrosine aminotransferase and tryptophan oxygenase activity in intact mice and in mice with hypothalamic lesions induced by GTG treatment. The induction of these enzymes may have been mediated by stimulation of adrenal medullary secretion.

Zusammenfassung. 2-Deoxyglukose führt bei Mäusen zu Blutzuckersteigerung und in der Leber zu Aktivierung der Tryptophan-Pyrrolase und Tyrosine-Transaminase bei durch Goldthioglukose verursachten hypothalamischen Schäden.

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[20] E. C. C. LIN, B. M. PITT, M. CIVEN and W. E. KNOX, J. biol. Chem. *233*, 668 (1958).
[21] W. E. KNOX and V. H. AUERBACH, J. biol. Chem. *214*, 307 (1955).
[22] L. YUWILER, L. WETTERBERG and E. GELLER, Biochem. biophys. Acta *208*, 428 (1970).
[23] R. W. FULLER and H. D. SNODDY, Biochem. Pharmac. *19*, 1518 (1970).
[24] L. RICHARDSON and B. HOKFELT, Proc. Soc. exp. Biol. Med. *117*, 83 (1964).
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Early and Selective Increase in Brain Dopamine Levels after Axotomy

The dopamine (DA) in the neostriatum (the caudate nucleus plus putamen) disappears within a week after making lesions in the cell bodies or in the non-terminal axons belonging to the nigro-neostriatal DA neurons[1-3]. This is probably due to degeneration and death of the DA-containing axon terminals of these neurons. Similarly, ascending noradrenaline (NA) neurons have been demonstrated and mapped out[2,4]. In this investigation, the time courses of the changes in the DA and NA concentrations of the forebrain have been studied both biochemically and histochemically in more detail, and have been compared in the same animals, after lesions were made in the ascending DA and NA pathways.

Material and methods. In the biochemical experiments, hooded rats of both sexes weighing about 200 g were used. Under pentobarbital sodium anaesthesia (about 40 mg/kg i.p.), a complete and almost frontal hemisection of the forebrain through the caudal hypothalamus was made by means of a blunt-edged spatula[5]. In the control rats, only the skull and dura were opened. At different time intervals after the operation, the rats were decapitated and the lesion was extended to the other side. The DA and NA on both sides of the forebrain frontal to the lesion were determined spectrophotofluorimetrically after cation exchange chromatography and oxidation[6-8].

[1] N.-E. ANDÉN, A. CARLSSON, A. DAHLSTRÖM, K. FUXE, N.-Å. HILLARP and K. LARSSON, Life Sci. *3*, 523 (1964).
[2] N.-E. ANDÉN, A. DAHLSTRÖM, K. FUXE, K. LARSSON, L. OLSON and U. UNGERSTEDT, Acta physiol. scand. *67*, 313 (1966).
[3] L. J. POIRIER and T. L. SOURKES, Brain *88*, 181 (1965).
[4] U. UNGERSTEDT, Acta physiol. scand., Suppl. 367 (1971).
[5] R. HASSLER and I. J. BAK, in Third Symposium on Parkinson's Disease (Eds. F. J. GILLINGHAM and I. M. L. DONALDSON; Livingstone Ltd., Edinburgh and London 1969), p. 29.
[6] A. CARLSSON and B. WALDECK, Acta physiol. scand. *44*, 293 (1958).
[7] A. CARLSSON and M. LINDQVIST, Acta physiol. scand. *54*, 87 (1962).
[8] J. HÄGGENDAL, Acta physiol. scand. *59*, 242 (1963).