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COMPARISON OF INSULIN HYPOGLYCEMIA-INDUCED AND FLUOROACETATE-INDUCED CONVULSIONS IN GOLD THIOGLUCOSE LESIONED MICE

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Abstract—In addition to its well known effects in producing hyperphagia, resulting in obesity, and histological damage focused relatively selectively in the ventromedial hypothalamic area, systemically administered gold thioglucose (GTG) also increased the sensitivity to insulin hypoglycemic convulsions. The change was in the convulsive response to equal hypoglycemia, rather than in the degree of hypoglycemia in response to insulin. The effect suggests that a relatively discrete control center is involved in adjusting brain function to metabolic alterations, in this case hypoglycemia. These results compare the effects of GTG lesions on insulin hypoglycemic- and fluoroacetate-induced convulsions. Gold thioglucose lesions did not alter the sensitivity to another metabolic convulsant, fluoroacetate, which blocks the Krebs cycle by blocking the conversion of citrate to isocitrate. Thus, although related in both cases to a shortage of available cellular fuel, the metabolic convulsions induced by insulin hypoglycemia and by fluoroacetate must be qualitatively different. Moreover, operation of the glucose sensing and regulatory mechanism lesioned by gold thioglucose, i.e. the "gold thioglucose lesioned glucostat", did not appear to require the Krebs cycle as blocked by fluoroacetate.

Systemic administration of gold thioglucose (GTG)§ to mice has long been known to produce hyperphagia and obesity and to be associated with histological damage focused relatively selectively in the ventromedial (VMH)-arcuate hypothalamic area [1–3]. Recently, it has been found that these cytotoxic lesions also alter the sensitivity to insulin hypoglycemic convulsions [4, 5, ‖]. The hypoglycemic challenge to these animals resulting from insulin is the same as in controls, so the difference must be in the convulsive response of the brain to hypoglycemia. This is quite striking because it suggests that a relatively discrete brain region is involved in controlling the generalized functional response of the brain to hypoglycemia.

As discussed elsewhere [5, ‖], the mechanism of GTG toxicity is not yet resolved. Regardless of this, the hyperphagia, obesity and altered sensitivity to insulin hypoglycemic convulsions all represent disturbances of glucoregulatory mechanisms and thus indicate the existence of a functional "GTG lesioned glucostat(s)" involved in the sensing and control of metabolic homeostasis. A GTG lesion may include some extrahypothalamic as well as VMH damage [2, 3, 6, 7], but no assumptions will be made as to whether the anatomical locus of the glucostat is at the focus of the main histological damage. Similarily, no assumptions will be made concerning whether these various functional changes are mediated by one or multiple but overlapping "GTG lesioned glucostat(s)".

Convulsions induced by both insulin and fluoroacetate are of metabolic origin. Insulin reduces the amount of glucose available to the brain by reducing it at the source (blood glucose) and hence it reduces all pathways of glucose oxidation, including both glycolysis and the Krebs cycle [8, 9]. Fluoroacetate, on the other hand, blocks the Krebs cycle specifically. Fluoroacetate undergoes a lethal synthesis to fluorocitrate which then blocks the enzyme aconitase and hence the conversion of citrate to isocitrate [10]. This metabolic block is at the level of high energy production (Fig. 1). The general assumption is that, in both cases, convulsions are due to the decreased availability of fuel supplies. We therefore thought it of interest to compare the effects of GTG lesions on these two metabolic convulsants. Metrazol-induced convulsions are generally considered to be independent of a metabolic block [11] and serve as a control for nonspecific effects on the response to stress or the generalized convulsive threshold and brain excitability.

MATERIALS AND METHODS

Female CBA/J mice from Jackson Laboratories (Bar Harbor, ME), a strain particularly susceptible to GTG toxicity, were used. Mice were fed Purina Laboratory chow *ad lib.* and housed in a room with a 12-hr on/12 hr off light-dark cycle. Fed mice were injected intraperitoneally with either GTG (0.8 mg/g), gold thiomalate (GTM) (0.8 mg/g), or *n*-saline (CS), all in volumes of 0.01 ml/g. GTG was

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§ Abbreviations: GTG, gold thioglucose; GTM, gold thiomalate; CS, *n*-saline control; and VMH, ventromedial hypothalamus.

‖ J. F. Holliday and M. A. Marrazzi, unpublished data.

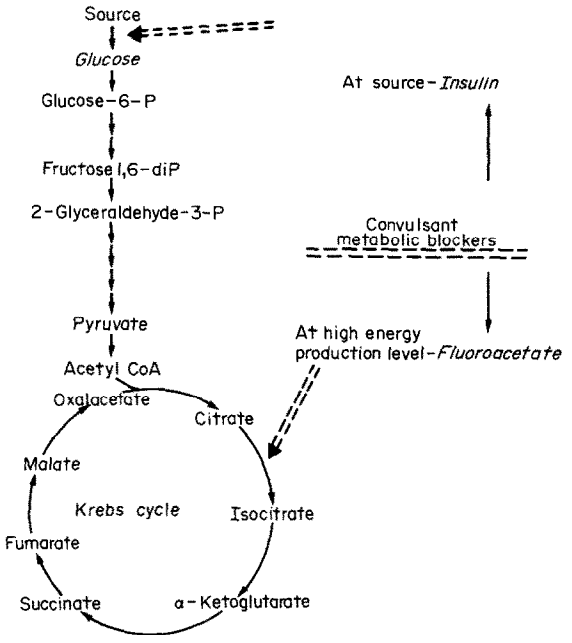


Fig. 1. Metabolic convulsants. Schematic diagram of glycolysis and Krebs cycle with arrows indicating the sites of action of insulin and fluoroacetate.

a generous gift from the Schering Corp. (Bloomfield, NJ), courtesy of Dr. Irving Tabachnick. GTM, used as a control for the non-specific effects of gold thio-compounds, was obtained from the Aldrich Chemical Co. (Milwaukee, WI).

The sensitivity to insulin hypoglycemic convulsions was tested as follows. Mice were fasted overnight, starting at 4:30 p.m., and tests were consistently run between 9:30 and 10:30 a.m. to control for variations in diurnal rhythm and duration of fasting. The mice were weighed and blood samples for glucose determination were taken just prior to insulin injection. These blood glucose values are referred to as "before". Insulin injections (2.5 mU/g, i.p. of Squibb regular insulin) were randomized among the three groups with respect to time, so that there was no time differential among the groups. The animals were then observed undisturbed and care was taken to prevent auditory and visual sensory input. When a mouse convulsed or at the end of 1 hr, whichever came first, a blood sample was immediately taken for glucose determination, and then glucose (50 mg, i.p.) was given to facilitate recovery. These blood glucose values are referred to as "after". Glucose effectively reversed the convulsions in both the lesioned and unlesioned groups. The times after insulin injection were followed for each mouse rather than taking an average for the entire group. By allowing this relatively limited 1-hr period in which the mice could convulse, we limited the range of hypoglycemia triggering the convulsions. If allowed additional time, more mice would have convulsed, but this would also have reflected still greater hypoglycemia at later times, and hence would not represent the same sensitivity to hypoglycemic convulsion. Thus, the percent convulsions was calculated as the percent of the mice per group that convulsed within 1 hr. Food was not available until after the hour was complete. Mice were then allowed to recover, were refed, and were then retested after 1 week or more for sensitivity to insulin or other convulsants. Repeated testing for convulsive activity separated by 1 week or more did not influence sensitivity to insulin hypoglycemic convulsions.

We have found that environmental conditions are critical in obtaining consistent results from day to day and from group to group. In addition to the factors mentioned above, we were careful not to disturb the mice and their diurnal rhythm of feeding the day before testing. Noise and activity in the animal room the day before were carefully avoided. Variations in the percent convulsions for a given dose of insulin may still be seen among different experiments, presumably due to unknown environmental variables. To be sure such factors did not affect our results, the conditions were carefully controlled among the different groups that were run simultaneously within each experiment.

The sensitivity to Metrazol-induced convulsions was tested in an analogous way to that for insulin. Metrazol (32.5 μg/g) was given intraperitoneally, and the anticonvulsant trimethadone (10 mg, i.p.) was given as the antidote. Although the mechanism of Metrazol-induced convulsions is not thought to be related to metabolic blockade, all the same variables pertaining to insulin, such as fasting and timing, were controlled.

Sensitivity to fluoroacetate-induced convulsions was tested in an analogous way. Fluoroacetate was given intraperitoneally in doses of 20 mg/kg. Unfortunately, no antidote is available for fluoroacetate-induced convulsions so that these tests were terminal.

Sodium monofluoroacetate (compound 1080) was obtained from the Tull Chemical Co. (Oxford, AL) as a technical grade compound which we then recrystallized. The purer commercially available preparations all contain a nigrosine dye, indicating presence of the rat poison. Our preparation did not. Recrystallization was achieved by dissolving the salt in analytical grade methanol with heat, filtering it gravimetrically, and then allowing it to cool and precipitate. This procedure was repeated several times.

Blood samples were obtained by cutting the tip of the tail with a scalpel and drawing 5 μl of blood from the tail vein into a disposable micropipet. This was then immediately delivered into a test tube and 50 μl of 0.6 N perchloric acid was added to precipitate protein. Samples were then neutralized with potassium bicarbonate. Glucose and citrate were assayed fluorometrically using hexokinase and citrate lyase respectively [12].

Histological damage was visualized with cresyl violet stain on frozen sections. Animals were perfused with 10% Lillie's buffered formalin through the heart.

Data on blood glucose and weight were analyzed using a simple one-way analysis of variance. Where appropriate, differences between means were examined with a Tukey B test. Data on percent convulsions were checked using a Chi^2 test [13].

Table 1. Effects of GTG lesions on insulin hypoglycemia-induced convulsions*

	GTG	GTM	CS
% Convulsions	96.9	31.3	46.9
Blood glucose (mM)			
Before insulin	3.89 ± 0.09	3.63 ± 1.0	3.26 ± 0.07
After insulin	0.95 ± 0.04	1.00 ± 0.04	0.96 ± 0.05
Body weight (g)	26.6 ± 1.3	19.1 ± 0.9	24.6 ± 0.5

* Mice were tested for the sensitivity to insulin hypoglycemic convulsions 10 days after treatment with gold thioglucose (GTG), gold thiomalate (GTM) or *n*-saline (CS). Initial body weights at the time of GTG, GTM or CS treatment were 26.5 ± 0.3 g, 27.0 ± 0.5 g and 26.1 ± 0.2 g respectively. The values are means ± S.E. $N = 32$. The statistically significant differences are as follows: %convulsions: GTG vs GTM or CS ($P < 0.01$); blood glucose before insulin: CS vs GTG or GTM ($P < 0.01$); and body weight: GTM vs GTG or CS ($P < 0.01$).

RESULTS

Table 1 shows that GTG-treated mice have a significantly higher percent convulsion in response to insulin than do controls treated with either *n*-saline or gold thiomalate. Gold thiomalate (GTM) is a control for the non-specific effects of a gold thiosalt. Since GTM behaves like unreactive gold thiosugars with respect to the hyperphagic and histological effects of GTG [14], it is commonly used in GTG studies as a control for the effects of gold thio-compounds in general. Although it is recognized that another gold thio-sugar might be a more discriminating control, none are commercially available.

The change in convulsive threshold occurred at a time when there was neglible difference in body weight between the GTG lesioned and saline control groups, so that the effect cannot be attributed to obesity. Blood glucose levels after insulin were the same in all three groups, i.e. the hypoglycemia resulting from insulin was the same. Therefore, the difference must be in the altered convulsive response of the brain to the hypoglycemic challenge. Blood glucose concentrations before insulin were slightly higher in the GTG lesioned mice than in controls. However, this occurred with the GTM-treated mice as well, indicating it was a nonspecific effect of gold thio-compounds.

Metrazol-induced convulsions were not altered by GTG lesions (Table 2). The percent convulsions in the GTG-lesioned and saline control groups were not significantly different. Although the percent convulsions in the GTM-treated group was lower, this is probably attributable to the lower body weight, so that the Metrazol dose on a per gram mouse basis results in less Metrazol per mouse. Both the GTG- and GTM-treated mice lost weight initially, due to the general toxicity of the gold thio-salts, and recovered back to normal. Since the GTG leisoned mice became hyperphagic, they lost less weight and recovered faster than the GTM controls. At this time, the GTG- but not the GTM-treated mice had returned to normal body weight. Thus, the GTM group must be interpreted with caution and in comparison with the saline-treated controls. GTG lesions are not altering the general non-specific convulsive threshold or the non-specific response to stress.

The sensitivity to fluoroacetate-induced convulsions was measured on a population of mice composed equally of those used to test the sensitivity to insulin hypoglycemic convulsions and those used to assess the sensitivity to Metrazol-induced convulsions. This was done so that the same or an overlapping population of mice could be tested with the different convulsants before the obesity of the GTG lesioned animals relative to the saline controls became a more marked significant complicating factor. Sequential testing of all three convulsants would have required a time span in which the weight gain

Table 2. Effects of GTG lesions on Metrazol-induced convulsions*

	GTG	GTM	CS
% Convulsions	68.8	12.5	53.1
Blood glucose (mM)			
Before Metrazol	4.75 ± 0.15	4.72 ± 0.08	3.72 ± 0.11
After Metrazol	5.10 ± 0.15†	5.37 ± 0.09	4.16 ± 0.19
Body weight (g)	26.7 ± 0.8	19.0 ± 0.2	24.3 ± 0.4

* Mice were tested for the sensitivity to Metrazol-induced convulsions 10 days after treatment with gold thioglucose (GTG), gold thiomalate (GTM), or *n*-saline (CS). Initial body weights at the time of GTG, GTM or CS treatment were 26.9 ± 0.3 g, 25.6 ± 0.7 g, and 25.0 ± 0.5 g respectively. The values are means ± S.E. $N = 32$ except as noted by the dagger (†), where $N = 31$. The statistically significant differences are as follows: % convulsions: GTM vs GTG or CS ($P < 0.01$); blood glucose before Metrazol: CS vs GTG or GTM ($P < 0.01$); blood glucose after Metrazol: CS vs GTG or GTM ($P < 0.01$); and body weight: GTG vs GTM vs CS ($P < 0.01$).

Table 3. Effects of GTG lesions on fluoroacetate-induced convulsions*

	GTG	GTM	CS
% Convulsions	40.6	48.4†	37.5
Blood glucose (mM)			
Before fluoroacetate	3.98 ± 0.06	3.35 ± 0.02†	3.36 ± 0.03
After fluoroacetate	3.28 ± 0.11†	3.60 ± 0.03†	3.19 ± 0.06‡
Body weight (g)	29.2 ± 1.0	21.9 ± 0.03§	24.3 ± 0.3

* Mice were tested for the sensitivity to insulin hypoglycemic convulsions 16 days after treatment with gold thioglucose (GTG), gold thiomalate (GTM) or *n*-saline (CS). These mice were composed of half the group tested with insulin in Table 1 and half the group tested with Metrazol in Table 2. After allowing recovery for 6 days, they were retested with fluoroacetate as presented here. The values are means ± S.E. $N = 32$ except as indicated by (†) where $N = 31$, (‡) where $N = 25$, or (§) where $N = 23$. The statistically significant differences are as follows: blood glucose before fluoroacetate: GTG vs GTM or CS ($P < 0.01$); and body weight: GTG vs GTM or CS ($P < 0.01$); GTM vs CS ($P < 0.05$).

Table 4. Blood citrate levels associated with fluoroacetate-induced convulsions*

	GTG	GTM	CS
Blood citrate (mM)			
Before fluoroacetate	1.51 ± 0.02 (32)	1.66 ± 0.01 (31)	1.80 ± 0.01 (32)
After fluoroacetate	5.58 ± 0.23 (31)	6.21 ± 0.35 (23)	6.56 ± 0.34 (25)

* These are the blood citrate levels (mM) determined in the experiment in Table 3. The values are means ± S.E. with N given in parentheses. There are no statistically significant differences among the three groups before fluoroacetate or among the three groups after fluoroacetate.

might have become a consideration. Since the fluoroacetate test is terminal, it had to be the last convulsant tested.

Table 3 shows that the threshold for fluoroacetate-induced convulsions, although due to metabolic blockade, was not altered by GTG lesions. Since the metabolic disturbance is at the level of the Krebs cycle [10], as expected, blood glucose was not affected. On the other hand, the blockade of the conversion of citrate was reflected in elevated blood citrate levels. Table 4 shows that the extent of the fluoroacetate block was the same in all three groups. The GTM- and saline-treated control groups were in better agreement in this experiment than in Table 2. Perhaps this was because body weight of the GTM group had returned to closer to the saline control group. Differences in the tissue distribution of Metrazol and fluoroacetate may also result in a difference in the dependence on the amount of body fat, i.e. the dose per gram body weight, and may be a factor.

Figures 2 and 3 show the histological damage from GTG in our studies.

DISCUSSION

GTG lesions increased the sensitivity to insulin hypoglycemic convulsions in these studies, rather than decreasing the sensitivity as previously reported by Kellar *et al.* [4]. The reason for this difference is not clear. We have found that control of environmental conditions is critical for achieving consistent results even between groups and this may play a role. Furthermore, as reported elsewhere [5], we have found that a decreased sensitivity may be seen at times prior to the increase reported here. Although according to our timing of the biphasic response [5] the 73 day post-lesion time period used by Kellar *et al.* would be expected to give a decreased threshold, there is indication that both changes may occur depending on the conditions. The mice in the study of Kellar *et al.* had about a 20 per cent weight gain over controls, which in our paradigm does not appear to be enough to produce an increased threshold due to the weight gain itself. One possibility is that there is a difference in the extent of the lesion in the two studies, independent of the weight gain. A great deal of variability among laboratories exists in the effectiveness of GTG. Thus, comparison of the dose between laboratories is difficult. The GTG dose in our study is twice that used by Kellar *et al.* (0.8 mg/g vs 0.4 mg/g). Caffyn [15] showed the spread of Evans blue 24 hr after GTG to be more extensive after a dose of 0.6 mg/g than of 0.4 mg/g. Perhaps the change in convulsive threshold and the hyperphagia are independent of each other and perhaps they do not correlate with the extent of the cytological damage in the same manner. It may be that the less extensive cytological damage postulated for 0.4 mg/g GTG never results in the component of the lesion responsible for the increased sensitivity. Or it may be that the time course for the second component (the increased sensitivity) at a lower GTG dose is much delayed and does not parallel the development of the weight gain. However, whatever the variable between these two studies, we have compared the sensitivity to insulin, fluoroacetate, and Metrazol-induced convulsions in the same popu-

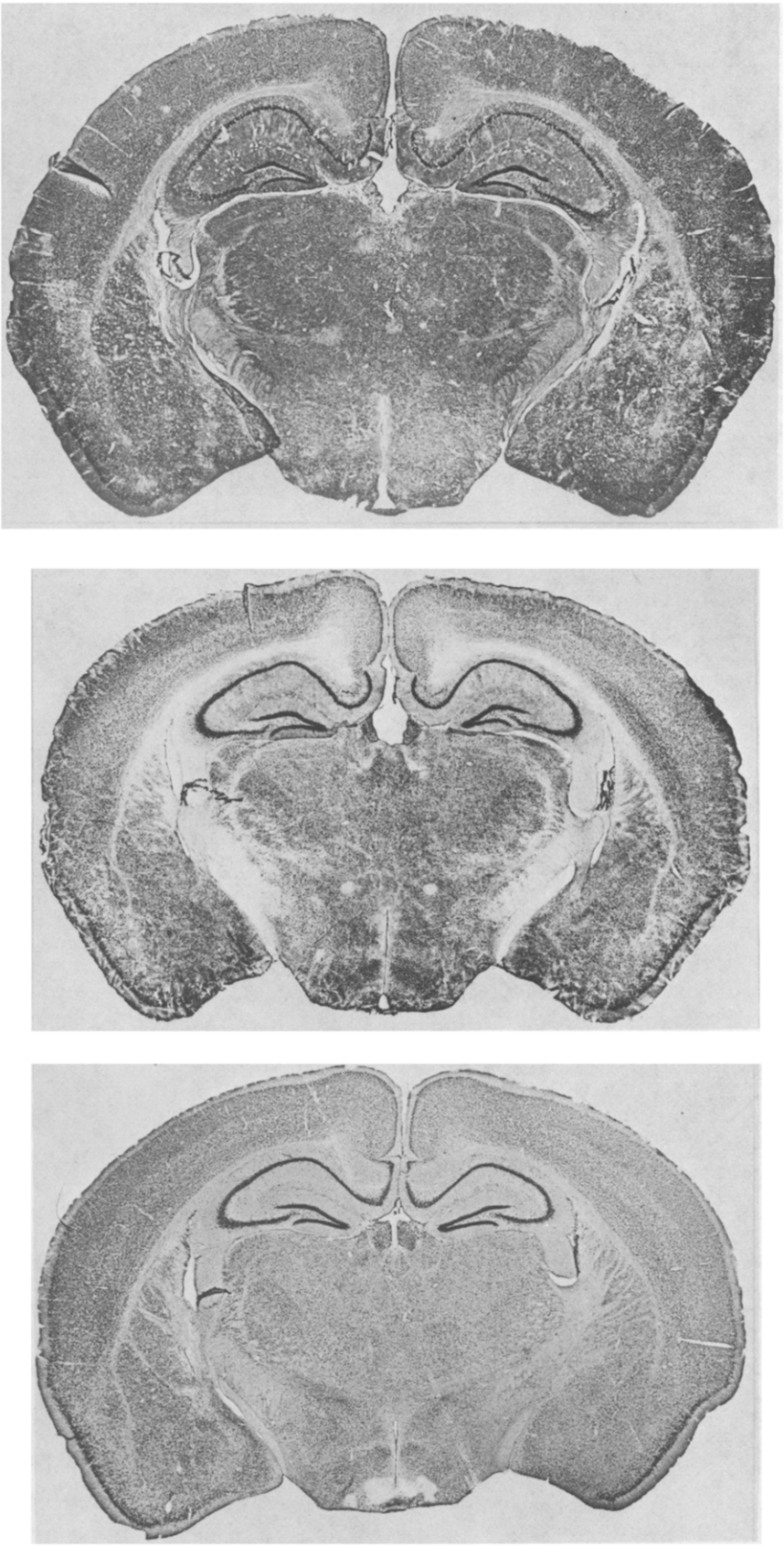
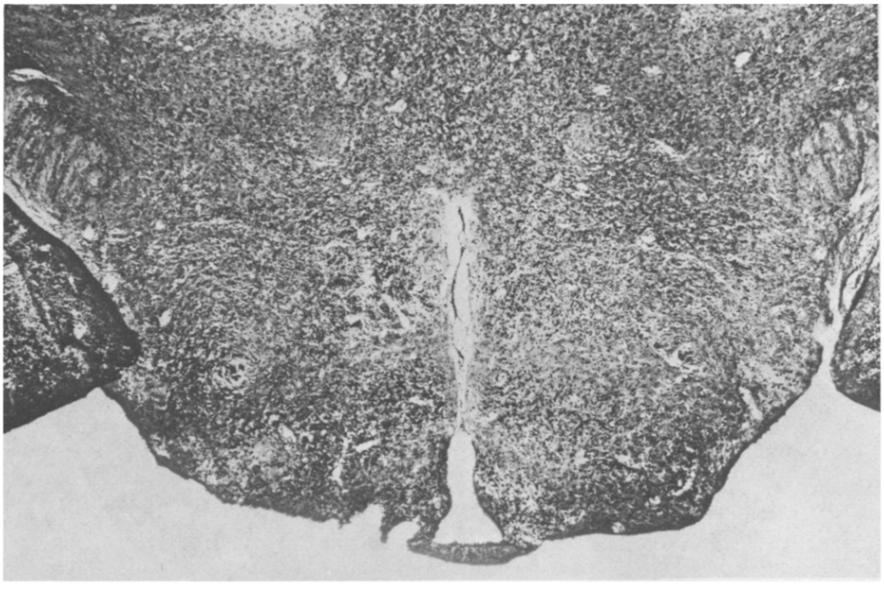
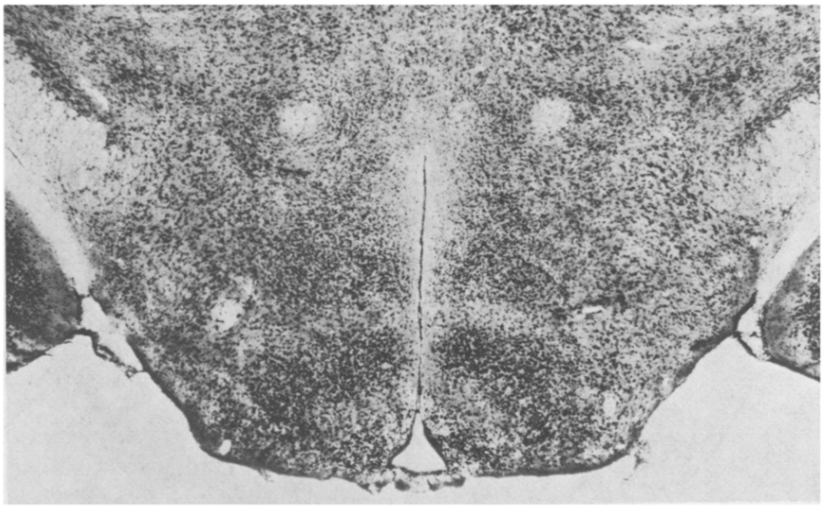


Fig. 2. GTG lesioned mouse brain. Cresyl violet stain, 10 × magnification, 40 μm thick sections. Key: (A) saline control, (B) 24 hr after GTM treatment, and (C) 24 hr after GTG treatment.

(A)



(B)



(C)

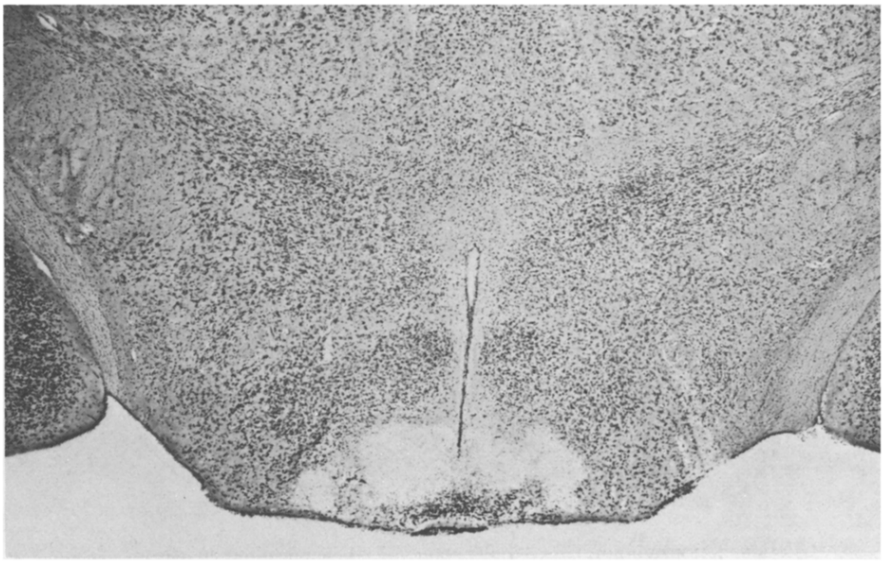


Fig. 3. GTG lesioned mouse brain. Same as in Fig. 2 but 30 × magnification.

lation of GTG lesioned animals under the same conditions in simultaneously run experiments.

Thus, GTG lesions alter the sensitivity to insulin hypoglycemia-induced but not to fluoroacetate-induced convulsions. Although having different biochemical mechanisms, both decrease the available cellular fuel and decrease energy metabolism. This, in turn, is generally considered to be the common denominator causing the common functional disturbance. However, the differential effects of GTG lesions indicate a qualitative difference in these two "metabolic convulsants". Moreover, fluoroacetate-induced convulsions do not need the "GTG lesioned glucostat".

These results also indicate that operation of the "GTG lesioned glucostat" does not require the Krebs cycle as blocked by fluoroacetate. Either the sensing mechanism does not depend on the level of metabolism or the rest of metabolism must be sufficient to maintain the sensing mechanism. It could be sensing the absolute level or the rate of decrease of glucose, as a measure of energy availability, or be affecting a nonmetabolic aspect of insulin hypoglycemic convulsions.

Two pools of the Krebs cycle exist in brain [16–19], but fluoroacetate blocks only the smaller compartment [17, 18]. The larger compartment may be included in the other aspects of metabolism possibly involved in maintenance of the "GTG lesioned glucostat", although convulsions from the fluoroacetate block are associated with the smaller compartment.

With regard to the metabolic mechanisms involved, it is also interesting to note that, despite the assumption that metabolic convulsions are due to a generalized power failure, this is not reflected in a generalized depletion of the high energy reserves. Despite the marked functional deficit of insulin hypoglycemic convulsion and even coma, ATP and glycogen are not markedly depleted and significant phosphocreatine levels are still present, when measured in relatively large brain regions [20–24]. Similarly, ATP and phosphocreatine are not changed in whole forebrain during fluoroacetate-induced convulsions [21]. Glycogen was not determined in this study. As we have discussed more fully elsewhere [5], this preservation of high energy levels might reflect a selective adjustment or rebalancing of brain function in response to these metabolic disturbances. The "GTG lesioned glucostat" may represent a relatively discrete brain region which is a control center involved in this adjustment. Recent preliminary electrophysiological data describing a cortical disinhibition associated with both insulin hypoglycemia- and fluoroacetate-induced convulsions [25, 26] further suggest a rebalancing of brain function as a mechanism in addition to or instead of a generalized power failure.

Although in some species the lethal effects of fluoroacetate are due to the cardiac toxicity rather than convulsions [27], using a crude 3-lead electrocardiogram, we did not find any cardiac changes in fluoroacetate-treated mice prior to the asphyxia of convulsion. This confirms that a central nervous system convulsant mechanism, rather than a cardiac mechanism, accounts for fluoroacetate toxicity in mice in these studies.

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REFERENCES

1. A. F. Debons, I. Krimsky, M. L. Maayan, K. Fani and F. A. Jimenez, *Fedn Proc.* **36**, 143 (1977).
2. P. M. Edelman, I. L. Schwartz, E. P. Cronkite and L. Livingston, *Ann. N.Y. Acad. Sci.* **131**, 485 (1965).
3. R. A. Leibelt and J. H. Perry, in *Handbook of Physiology* (Ed. C. F. Code), Vol. 1, Section 6, p. 271. American Physiology Society, Washington, DC (1967).
4. K. J. Kellar, A. E. Langley, B. H. Marks and J. J. O'Neill, *Science* **187**, 746 (1975).
5. J. L. Luby, J. F. Holliday, K. W. Fish and M. A. Marrazzi, *J. Pharmac. exp. Ther.*, in press.
6. G. Breacher, G. L. Laqueur, E. P. Cronkite, P. M. Edelman and I. L. Schwartz, *J. exp. Med.* **121**, 395 (1965).
7. J. H. Perry and R. A. Liebelt, *Proc. Soc. exp. Biol. Med.* **106**, 55 (1961).
8. T. E. Duffy and F. Plum, in *Basic Neurochemistry* (Eds. G. J. Siegel, W. R. Albers, R. Katzman and B. Agranoff), p. 646. Little, Brown & Co., Boston (1976).
9. L. Sokoloff, in *Basic Neurochemistry* (Eds. G. J. Siegel, R. W. Albers, R. Katzman and B. Agranoff), p. 388. Little, Brown & Co., Boston (1976).
10. R. A. Peters, *Biochemical Lesions and Lethal Syntheses.* p. 88. Macmillan, New York (1963).
11. D. N. Franz, in *The Pharmacological Basis of Therapeutics* (Eds. L. S. Goodman A. G. Gilman and B. G. Koelle), 5th Edn. p. 359. Macmillan, New York (1975).
12. O. H. Lowry and J. V. Passonneau, *A Flexible System of Enzymatic Analysis.* Academic Press, New York (1972).
13. B. J. Winer, *Statistical Principles in Experimental Design.* McGraw-Hill, New York (1971).
14. J. Mayer and N. Marshall, *Nature, Lond.* **178**, 1399 (1956).
15. Z. E. Caffyn, *J. Path.* **106**, 49 (1972).
16. S. C. Cheng, in *Metabolic Compartmentation in the Brain* (Eds. R. Balazs and J. E. Cremer), p. 107. John Wiley, New York (1972).
17. D. D. Clarke and S. Berl, in *Metabolic Compartmentation in the Brain* (Eds. R. Balazs and J. E. Cremer), p. 97. John Wiley, New York (1972).
18. J. E. Cremer, in *Metabolic Compartmentation in the Brain* (Eds. R. Balazs and J. E. Cremer), p. 81. John Wiley, New York (1972).
19. C. J. Van den Berg, in *Metabolic Compartmentation in the Brain* (Eds. R. Balazs and J. E. Cremer), p. 137. John Wiley, New York (1972).
20. J. A. Ferrendelli and M. M. Chang, *Archs. Neurol. Chicago* **28**, 173 (1973).
21. N. D. Goldberg, J. V. Passonneau and O. H. Lowry, *J. biol. Chem.* **241**, 3997 (1966).
22. L. J. King, O. H. Lowry, J. V. Passonneau and V. Venson, *J. Neurochem.* **14**, 599 (1967).
23. O. H. Lowry, in *Brain Work: The Coupling of Function, Metabolism and Blood Flow in Brain* (Eds. D. H. Ingvar and N. A. Lassen), p. 48. Munksgaard, Copenhagen (1975).
24. M. Tarr, D. Brada and F. E. Samson, *Am. J. Physiol.* **203**, 690 (1962).
25. W. Raabe, *Neurosci. Abstr.* **4**, 443 (1978).
26. W. Raabe and L. Zieve, *Neurosci. Abstr.* **3**, 144 (1977).
27. M. B. Chenoweth, *Pharmac. Rev.* **1**, 383 (1949).