Diabetologia 12, 263–267 (1976)

Diabetologia

The Comparative Effects of Barbituric Acid and Phenobarbital on Blood Glucose and Insulin Secretion in Mice*

J. H. Mennear, C. Schonwalder and E. T. Yau

Department of Pharmacology and Toxicology School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana, USA

Summary. The effects of barbituric acid and phenobarbital upon carbohydrate metabolism in mice were compared. An intraperitoneal dose of 100 mg/kg of barbituric acid increased blood glucose concentrations during an intravenous glucose tolerance test, but did not alter the rate of glucose disappearance from the blood. Barbituric acid also antagonized the hypoglycemic effect of intravenously administered tolbutamide. The same dose of phenobarbital had no effect. An in vitro concentration of 100 µg/ml of barbituric acid decreased the responsiveness of isolated mouse pancreatic islets to glucose stimulation (3.0 mg/ml D-glucose). Again phenobarbital, 100 µg/ml, was without effect. The structural similarities between barbituric acid, tolbutamide and alloxan suggest that the effects observed in these experiments might reflect a competition for binding to reactive sites on or within the pancreatic B-cell.

Key words: Barbituric acid, phenobarbital, tolbutamide glucose tolerance, isolated pancreatic islets, insulin release.

Pretreatment of rats or dogs with barbituric acid affords protection against the diabetogenic effect of alloxan [1]. Diphenylhydantoin (DPH) prevents the pancreotoxic action of alloxan [2] and has also been found to decrease glucose tolerance and to block the hypoglycemic action of tolbutamide in mice [3].

The demonstration of the in vivo interactions between DPH, alloxan and tolbutamide has led to the hypothesis that these agents compete for binding sites on the pancreatic B-cell membrane [4]. Evidence in support of this hypothesis, however, is indirect and does not rule out the possible role of the known membrane stabilizing action of DPH.

The experiments reported in this communication were conducted to determine if barbituric acid, like DPH, decreases glucose tolerance and antagonizes the hypoglycemic effect of tolbutamide in mice. Barbituric acid is closely related to alloxan in chemical configuration, but it has never been reported to stabilize biological membranes. Phenobarbital was included in these experiments because it is a barbituric acid derivative which possesses CNS depressant properties and stabilizes biological membranes.

Materials and Methods

a. Animals and Chemicals

Male Swiss albino mice (Laboratory Supply Company, Indianapolis, IN) weighing 20 to 30 g were used in all experiments. The animals were housed in groups of ten with free access to food and water. The mice were acclimatised to laboratory conditions for one week prior to experimentation.

Barbituric acid (98% purity, Aldrich Chemical Co.) and sodium phenobarbital (98% purity, Merck) were dissolved in saline immediately before use. D-glucose (Mallinckrodt) was dissolved in distilled water 24 h prior to use and tolbutamide sodium (Upjohn) was dissolved in pH 8.3 Tris buffer (50 mM). Regular bovine insulin (Lilly) was diluted with saline just prior to use. All solutions and suspensions were prepared at concentrations which allowed for the administration of volume doses of 10 ml/kg. Barbituric acid and phenobarbital were administered intraperitoneally, glucose and tolbutamide intravenously and insulin subcutaneously.

* This research was supported by grants CA 13285 and AM 14134 from the National Institutes of Health.

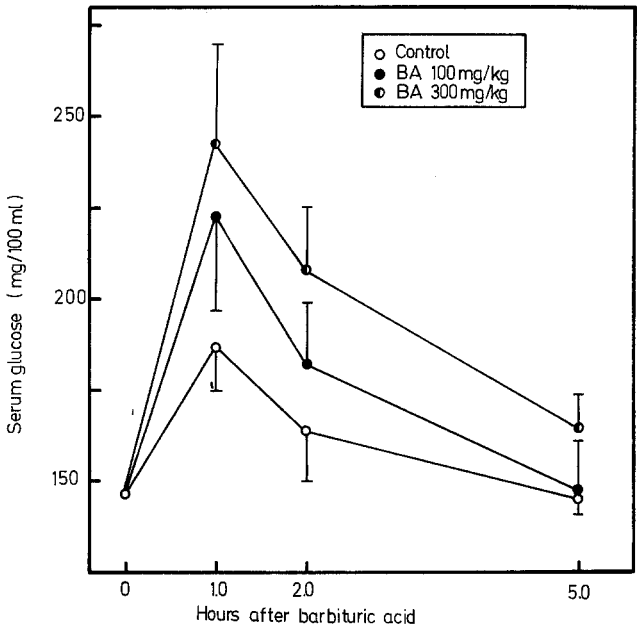

Fig. 1. Effect of barbituric acid (BA) on serum glucose concentration in mice. Each point represents the mean serum glucose value of five mice. Vertical bars represent the SEM. The 100 mg/kg dose of BA did not produce significant hyperglycemia. Mean serum glucose concentrations were significantly elevated by the 300 mg/kg dose of BA ($p < 0.05$)

b. Glucose Tolerance Test

Mice were pretreated with 100 mg/kg of either barbituric acid or phenobarbital 60 min before the intravenous injection of 2.0 gm/kg of D-glucose. This high dose of glucose was found to be necessary in order to sustain elevated serum glucose concentrations.

Serum glucose concentrations were determined 15, 30, 60 120 and 150 min after glucose injection. The rate of glucose disappearance (K) was determined as described by Conard [5] from the formula K (%/min) $= \frac{69.3}{t^1/_2}$, where $t^1/_2$ is the time, in minutes, for the glucose concentration to fall from the value at 15-minutes to one-half of that value. Glucose distribution space was estimated by the method of Ganda et al. [6].

c. Tolbutamide and Insulin Injection

Mice were pretreated with 100 mg/kg of either barbituric acid or phenobarbital 60 min before the administration of either 75 mg/kg of tolbutamide or 0.25 unit of insulin. Serum glucose concentrations were determined immediately before and at several intervals after the administration of the hypoglycemic agents.

d. In Vitro Experiments

Pancreatic islets were isolated by the collagenase method of Lacy and Kostianovsky [7]. Immediately after isolation the islets were incubated for 30 min at 37° in Krebs-Ringer bicarbonate buffer supplemented with 0.3% bovine serum albumin and 0.6 mg/ml of D-glucose. After stabilization, groups of three islets were incubated for 90 min under an atmosphere of 95% oxygen and 5% carbon dioxide in 1.0 ml of fresh medium containing 100 μg/ml of either barbituric acid or phenobarbital and 3.0 mg/ml of D-glucose. Incubations were carried out in a Dubnoff metabolic shaker (60 oscillations/min).

e. Glucose and Insulin Assays

Blood samples were obtained by orbital sinus puncture and serum glucose concentrations were determined by a glucose oxidase method (Beckman Glucose Analyzer).

Aliquots (0.1 ml) of the islet incubation medium were taken at 30 min intervals and assayed for immunoreactive insulin (IRI) using a commercial kit (Amersham/Searle). Human insulin was used as the insulin standard.

f. Statistical Analyses

Differences between mean serum glucose concentrations and glucose disappearance rates were assessed for significance by the use of Student's t test. The effects of barbituric acid and phenobarbital on glucose-stimulated insulin release from isolated pancreatic islets were compared by one way analysis of variance.

Results

a. Effect of Barbituric Acid and Phenobarbital on Serum Glucose Concentration

The results presented in Figure 1 show that the 100 mg/kg dose of barbituric acid produced an insignificant rise in serum glucose concentration. The 300 mg/kg dose produced significant hyperglycemia, which persisted for at least two hours.

Phenobarbital, at a dose of 100 mg/kg, had no effect. A higher dose of 300 mg/kg was lethal within three hours, so a direct comparison with barbituric acid was not possible.

b. Effect of Barbituric Acid and Phenobarbital on Intravenous Glucose Tolerance

The 100 mg/kg dose of barbituric acid resulted in a significant elevation of serum glucose concentrations

15, 30 and 60 min after the intravenous injection of glucose (Tab. 1). The same dose of phenobarbital, however, was without effect. As shown in Table 1, when glucose disappearance rates were estimated it was found that either barbituric acid or phenobarbital had no effect on the glucose disappearance rate, although barbituric acid produced a marked decrease in the glucose distribution space (7.68 ± 0.4 ml compared with 12.6 ± 1.2 ml in control mice).

c. Effect of Barbituric Acid and Phenobarbital on the Hypoglycemic Effect of Tolbutamide

The results shown in Table 2 demonstrate the pronounced effect of tolbutamide on serum glucose concentrations in control mice. A significant hypoglycemia developed within 30 min and persisted throughout the remainder of the experimental period. When tolbutamide was administered to barbituric acid pretreated mice, however, no hypolgycemia was observed. Pretreatment with phenobarbital, on the other hand, did not alter the magnitude of tolbutamide-induced hypoglycemia.

d. Effect of Barbituric Acid on the Hypoglycemic Effect of Insulin

Pretreatment of mice with 100 mg/kg barbituric acid one hour before the administration of insulin did not antagonize the hypoglycemic effect of the hormone. One hour after insulin administration mean decreases in serum glucose of 102 and 107% were observed.

e. Effect of Barbituric Acid and Phenobarbital on Glucose-Stimulated Insulin Release from Isolated Mouse Pancreatic Islets

The results of this experiment are shown in Table 3. Control islets exhibited a steady release of IRI during the entire incubation period, while barbituric acid exposed islets released detectable quantities of IRI during only the 30 and 60 min intervals. No release was observed between 60 and 90 min. Over the entire incubation period IRI release by the barbituric acid exposed islets was only 40% of that released by controls ($p < 0.01$). Phenobarbital, on the other hand, did not significantly reduce glucose-stimulated IRI release during any of the time intervals tested.

Discussion

The effects of barbituric acid reported in this communication are similar to those which have been noted earlier for diphenylhydantoin [2, 3, 8, 9]. Both chemicals increase serum glucose concentrations during the glucose tolerance test, antagonize the hypoglycemic effect of tolbutamide and inhibit glucose-induced IRI release *in vitro*.

The structural similarities between alloxan, barbituric acid, tolbutamide and diphenylhydantoin have led to an earlier suggestion that the observed interactions may be reflections of competition for binding sites on the pancreatic B-cell membrane [1, 4]. All of these chemicals possess unsubstituted ureido groups in their structural configurations and the importance of this portion of the molecules of alloxan and diphenylhydantoin have been shown in earlier in vivo experiments [4, 10].

The fact that DPH is known to stabilize biological membranes suggests that the pancreatic effect of the anticonvulsant may be a nonspecific reflection of this action. Stabilization of the B-cell membrane would be expected to inhibit both glucose- and tolbutamide-stimulated insulin secretion. Also, since alloxan has been shown to produce an irreversible depolarization of the B-cell membrane [11], stabilization of the membrane might explain DPH antagonism of the diabetogen.

It is unlikely, however, that membrane stabilization can account for the pancreatic effects of barbituric acid. Barbituric acid does not produce central nervous system depression and, to our knowledge, has never been reported to stabilize biological membranes.

Although our experiments demonstrate a barbituric acid-induced inhibition of glucose stimulated insulin release from isolated pancreatic islets, it is not possible to conclude that the in vivo effects of the chemical on carbohydrate metabolism are mediated through this mechanism alone. It is unlikely that barbituric acid antagonizes the peripheral action of insulin since the chemical did not reduce insulin-induced hypoglycemia in our mice.

Since phenobarbital possesses the unsubstituted ureido group and is known to stabilize biological membranes, its failure to influence the parameters studied in these experiments was surprising. These results are in agreement with those of Japundzic et al. [12] and Sato and Iwamoto [13] who reported that phenobarbital had no effect on blood glucose concentrations in rats and Gerhards et al. [14], who reported that the drug has no effect on the hypoglycemic response to glycodiazine in rats, dogs or humans.

Malygina [8], however, presented data to show that phenobarbital produces hypoglycemia in rabbits, while Stevens et al. [15] reported decreased glucose tolerance in rats. Yeung [16] observed phenobarbital-induced *hyperglycemia* in new born humans.

Our discovery of the effects of barbituric acid on carbohydrate metabolism suggests a potential clinical

Table 1. Effect of barbituric acid and phenobarbital on glucose tolerance in mice[a]

Treatment	Serum glucose (mg/100 ml) ± SEM at time intervals (min) after glucose					
	0[b]	15	30	60	90	K (%/min)
Control	544 ± 37	510 ± 30	438 ± 42	346 ± 44	317 ± 38	0.68 ± 0.12
Barbituric acid	777 ± 33[c]	707 ± 22[c]	584 ± 29[c]	568 ± 62[c]	372 ± 35	0.86 ± 0.12
Control	382 ± 22	395 ± 19	260 ± 19	211 ± 21	202 ± 18	0.82 ± 0.13
Phenobarbital	464 ± 22	444 ± 15	327 ± 28	257 ± 23	219 ± 19	0.91 ± 0.07

[a] Mice (5 per group) were treated with 100 mg/kg, i.p. of either barbituric acid or phenobarbital one hour prior to the i.v. administration of 2.0 gm/kg of D-glucose.
[b] Values presented at 0 min are estimates derived from the semilog plot employed to estimate glucose disappearance rate (K).
[c] Significantly greater than control ($p < 0.05$).

Table 2. Effect of barbituric acid and phenobarbital on the hypolgycemic action of tolbutamide in mice[a]

Treatment	Serum glucose (mg/100 ml) ± SEM at time intervals (min) after tolbutamide				
	0	30	60	90	240
Saline + tolbutamide	163 ± 3	93 ± 4	– –	107 ± 4	127 ± 5
Barbituric acid + tolbutamide	166 ± 3	166 ± 15[b]	– –	168 ± 17[2]	188 ± 13[b]
Saline + tolbutamide	150 ± 11	99 ± 7	98 ± 13	86 ± 11	– –
Phenobarbital + tolbutamide	144 ± 12	104 ± 9	91 ± 10	86 ± 10	– –

[a] Mice (5 per group) were pretreated with either saline, barbituric acid (100 mg/kg, i.p.) or phenobarbital (100 mg/kg, i.p.) one hour prior to the i.v. administration of 75 mg/kg tolbutamide.
[b] Significantly greater than the corresponding saline pretreated control ($p < 0.05$).

Table 3. Effect of barbituric acid and phenobarbital on the responsiveness of isolated mouse pancreatic islets to D-glucose

	Mean μU-IRI/islet ± SEM at time of incubation (min)			
	0	30	60	90
Control	5.3 ± 1.1	14.1 ± 2.9	19.9 ± 2.3	39.9 ± 5.8*
Barbituric acid[a]	5.7 ± 0.7	12.3 ± 3.3	16.1 ± 3.6	15.9 ± 2.9
Control	8.1 ± 1.2	16.5 ± 1.8	18.8 ± 2.8	39.9 ± 5.7
Phenobarbital[b]	10.0 ± 1.1	14.9 ± 0.7	17.3 ± 1.5	32.2 ± 4.1

[a] Six sets of isolated islets (3 per set) were exposed to 100 μg/ml barbituric acid and 3.0 mg/ml D-glucose. *A significant difference was detected at the 90 min interval ($p < 0.01$).
[b] Four sets of isolated islets (3 per set) were exposed to 100 μg/ml phenobarbital and 3.0 mg/ml D-glucose. No significant differences in IRI release were noted at any time interval.

use for this chemical. Several therapeutic agents, such as the thiazide diuretics and diphenylhydantoin, are known to inhibit pancreatic insulin secretion. This action, which was initially considered to be an undesirable side-effect, has been employed in the treatment of hypoglycemia. The obvious disadvantage of these drugs lies in the fact that they possess well known pharmacological activities which, when the drugs are employed in the treatment of hypoglycemia, may be considered to be undesirable side-effects. Barbituric acid appears to be devoid of effects other than those on carbohydrate metabolism. We believe that further investigations into the potential use of barbituric acid in the treatment of functional hypoglycemia are warranted.

References

1. Martinez, C.: Effect of barbituric acid on alloxan diabetes in rats and dogs. Amer. J. Physiol. **182**, 267–268 (1955)
2. Mennear, J. H., Gossel, T. A.: Inhibitory effect of diphenylhydantoin on the diabetogenic action of alloxan in the mouse. Diabetes **21**, 80–83 (1972)
3. Mennear, J. H., Gossel, T. A.: Interactions between diphenylhydantoin and tolbutamide in mice. Toxicol. appl. Pharmacol. **24**, 309–316 (1973)
4. Exposito-Avella, M., Mennear, J. H.: Studies on the protective effect of diphenylhydantoin against alloxan diabetes in mice. Proc. Soc. exp. Biol. (N.Y.) **142**, 82–85 (1973)
5. Conard, V.: Mesure de l'assimilation du glucose. Acta gastroent. belg. **18**, 655–705 (1955)
6. Ganda, O. P., Kahn, C. B., Soeldner, J. S., Gleason, R. E.: Dynamics of tolbutamide, glucose, and insulin interrelationships following varying doses of intravenous tolbutamide in normal subjects. Diabetes **24**, 354–361 (1975)
7. Lacy, P. E., Kostianovsky, M.: Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes **16**, 35–39 (1967)
8. Malygina, E. I.: Effects of phenobarbital, diphenylhydantoin and trimethadione on blood sugar level. Farmakol. i. Toksikol. **28**, 725–727 (1965)
9. Kizer, J. S., Vargas-Cordon, M., Brendel, K., Bressler, R.: The in vitro inhibition of insulin secretion by diphenylhydantoin. J. clin. Invest. **49**, 1942–1948 (1970)
10. Bruckmann, G., Wertheimer, E.: Alloxan studies. The action of alloxan homologues and related compounds. J. biol. Chem. **168**, 241–256 (1947)
11. Dean, P. M., Matthews, K. E.: The bioelectric properties of pancreatic islet cells. Effects of diabetogenic agents. Diabetologia **8**, 173–178 (1972)
12. Japundzic, I., Japundzic, M., Knezevic, B., Mimic-Oka, J.: The influence of phenobarbital on the glycogen concentration of rat liver. Experientia (Basel) **25**, 478–479 (1969)
13. Sato, T., Iwamoto, T.: Neurotrophic drugs, electroshock and carbohydrate metabolism in the rat. Biochem. Pharmacol. **15**, 323–331 (1966)
14. Gerhards, E., Kolb, K. H., Schulze, P. E.: The metabolism of glycodiazine and tolbutamide after phenobarbital treatment. Naunyn-Schmiedeberg's Arch. Pharmacol. **255**, 200–220 (1966)
15. Stevens, C. D., Herzog, W., Turner, M. E.: production of temporary prolonged hyperglycemia by the administration of glucose with barbiturates and sulfapyrazine. J. Pharmacol. exp. Ther. **141**, 267–273 (1963)
16. Yeung, C. Y.: Blood sugar changes in neonatal hyperbilirubinaemia and phenobarbitone therapy. Arch. Dis. Childh. **47**, 246–249 (1972)

Received: September 15, 1975, and in revised form: April 6, 1976

Dr. J. H. Mennear
Dept. of Pharmacology and Toxicology
School of Pharmacy and Pharmacal Sciences
Purdue Univ.
West Lafayette, Indiana 47907
USA