

The Effects of Selected Barbituric Acid Analogues on Glycemic Responses in Mice¹ (40427)

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Structure-activity relationship studies by Brückmann and Wertheimer (1) have assessed the structural components required for alloxan to elicit its pancreatotoxic qualities. These workers reported that substitutions either in *both* imino groups of the ureido moiety or in the C-5 position of the pyrimidine nucleus abolished the diabetogenic properties of the compound. Furthermore, they found that the diabetogenicity of alloxan was reduced as the length of a single aliphatic side chain on one imino group was increased. Later, Griffiths (2) confirmed that the unsubstituted ureido moiety of alloxan was required to produce diabetes.

In 1955, Martinez (3) demonstrated that rats and dogs were protected against the diabetogenic activity of alloxan when the animals were pretreated with another urea-derived chemical, barbituric acid (BA). Recently, Mennear and colleagues (4) have reported that BA reduces glucose tolerance, antagonizes the hypoglycemic effects of tolbutamide, and inhibits D-glucose-stimulated insulin release directly from isolated pancreatic islets. More recent investigations by Wright and Mennear (5) have demonstrated that although BA elicits a dose-dependent hyperglycemia following intraperitoneal administration, adrenalectomy blocked this response. In addition, these workers reported that BA attenuation of tolbutamide-induced hypoglycemia was not blocked by adrenalectomy which further suggested a direct inhibition of pancreatic β cell activity. Since

BA differs structurally from alloxan only by a carbonyl group in the C-5 position, it was the objective of this investigation to assess several analogues of BA with substitutions on the ureido group to ascertain the importance of this moiety in affecting glycemic homeostasis.

Materials and methods. Swiss Webster-derived male mice (Laboratory Supply Co., Indianapolis) weighing 25–30 g were housed in groups of 10 with free access to food (Wayne Lab-Blox, Allied Mills, Inc., Chicago) and water. When adrenalectomized mice were employed, the animals were maintained post-operatively for 66 hr on laboratory food and 0.9% NaCl drinking fluid until experimentation.

Barbituric acid (Aldrich Chemical Co.), 2-thiobarbituric acid (Eastman Kodak Co.), 1-methyl barbituric acid and 1,3-dimethyl barbituric acid (Heterocyclic Chemical Co.) were dissolved in redistilled water immediately prior to intraperitoneal injection of 200 mg/kg (20 ml/kg). Because of the acidity of the solutions of the BA derivatives, the redistilled water administered to the control animals was adjusted to pH 2.4. In the glucose tolerance experiments, D-glucose (Mallinckrodt Chemical Works) was prepared in redistilled water 24 hr before use to allow for complete mutarotation and administered intraperitoneally (2.0 g/kg; 10 ml/kg) 30 min after the appropriate pretreatment.

Repeated blood samples were obtained from each animal by orbital sinus puncture (6). Serum glucose concentrations were determined by a glucose oxidase method (Beckman Glucose Analyzer) and expressed as mg of glucose per 100 ml of serum.

The results of each experiment were compared statistically using a two factor analysis of variance for repeated measures of samples and compared to resting levels and/or control values by the method of Dunnett (7).

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Results. Previous studies from our laboratories have demonstrated that the intraperitoneal administration of BA elicits a dose-dependent hyperglycemic response in both mice (4) and rats (5). The results presented in Fig. 1 confirm these reports using doses of BA of 50, 100, and 200 mg/kg. Although moderate hyperglycemia was observed in the control group throughout the initial 120 min of the experiment, serum glucose concentrations in these animals were not significantly greater than pretreatment levels at any interval tested. In addition, the 50 mg/kg dose of BA failed to alter serum glucose concentrations significantly from control values. The group receiving 100 mg/kg of BA demonstrated elevated serum glucose levels throughout the experiment, peaking at 60 min after injection and attaining a level significantly different from control only at 180 min after treatment. However, the 200 mg/kg dose of BA produced a significant hyperglycemia at each of the post-injection periods peaking at 60 min and persisting throughout the duration of the experiment. Interestingly, although BA serves as the parent structure of several sedative-hypnotic agents, this property was not observed after the administration of either BA or the analogues.

Because the intraperitoneal administration of BA elicited a dose-dependent hyperglycemia, and in order to establish a dose range for the subsequent glucose tolerance tests, a dose-dependent relationship to this response was investigated using 1-methyl BA, 1,3-dimethyl BA, and 2-thio BA. It can be seen from the results in Table I that the control animals consistently exhibited moderate hyperglycemic responses. However, in contrast to the response elicited with BA administration, no significant elevations in serum glucose concentrations above control were observed at any interval with the 100 or 200 mg/kg doses of 1-methyl BA, the 50, 100, or 200 mg/kg doses of 1,3-dimethyl BA, or the 100 mg/kg dose of 2-thio BA. Only the 200 mg/kg dose of 2-thio BA produced a significantly elevated serum glucose concentration which returned to control levels by 45 min. These results indicate that substitutions on the ureido moiety of the BA molecule depress the ability of the compound to elicit a hyperglycemic response.

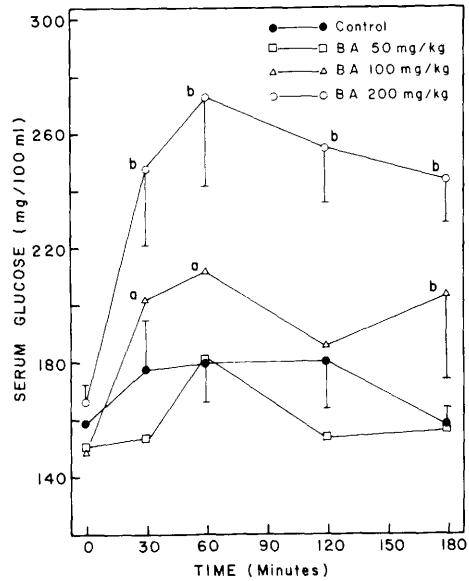


FIG. 1. Serum glucose concentrations in mice following barbituric acid administration. Each point represents the mean serum glucose concentration from five free-feeding mice at various intervals after injection. Vertical bars represent the SEM (some bars have been omitted for clarity). ^a Significantly greater ($p < 0.05$) than pretreatment value (i.e., 0 min). ^b Significantly greater ($p < 0.05$) than pretreatment value and control at same time interval.

Earlier it was reported that BA administration decreased glucose tolerance by diminishing pancreatic insulin secretory activity (4). Since glucose is a normal physiologic stimulus for insulin release, the glycemic effects of pretreatment with each of the BA analogues on glucose tolerance was investigated. The results of this experiment, presented in Table II, demonstrate that moderate but not significant hyperglycemic responses were observed in all groups 30 min after pretreatment with BA or a derivative. In the 15- and 30-min intervals after glucose challenge, serum glucose concentrations significantly greater than control were noted in all but the 1,3-dimethyl BA pretreated animals. These animals demonstrated a glucose intolerance significant from control only at the 45-min interval. However, by 60 min only the BA pretreated mice exhibited a hyperglycemic response greater than control. These results indicate that BA pretreatment reduced glucose tolerance for over 60 min while pretreatment with the BA derivatives with substitutions on the

ureido group actually diminished the magnitude and duration of the glucose intolerance when compared to the response elicited with the parent compound.

Because of irritant properties associated with the intraperitoneal administration of BA and its derivatives, the possibility of stress-related factors contributing to the hyperglycemic responses observed with these compounds was of interest. One effect of stress is the release of epinephrine from the adrenal medulla which, by itself, inhibits insulin release and stimulates hepatic glycogen breakdown, both actions promoting increased serum glucose concentrations. In order to in-

vestigate the effects of stress-related factors on the glycemic responses, the glucose tolerance test in animals pretreated with either BA or its derivatives was repeated using mice which had been adrenalectomized 66 hr earlier.

It can be seen from Table III that adrenalectomy appreciably attenuated the initial hyperglycemia which was observed 30 min after pretreatment of the intact mice with either BA or one of its derivatives. Although glucose intolerance was noted at the 15-min interval with the BA and the 1-methyl BA pretreated animals, only those adrenalectomized mice receiving BA pretreatment demonstrated glu-

TABLE I. SERUM GLUCOSE CONCENTRATIONS IN MICE FOLLOWING ADMINISTRATION OF BARBITURIC ACID ANALOGUES^a.

Treatment	Serum glucose concentration (mg/100 ml ± SEM) Time (min)					
	0	15	45	90	120	180
Control	190.8 ± 14.2	280.0 ± 25.0 ^b	288.0 ± 36.4 ^b		231.5 ± 33.0	
1-Methyl Barbituric Acid (100 mg/kg)	164.3 ± 9.3	217.0 ± 17.3 ^b	234.8 ± 28.0 ^b		191.3 ± 15.2	
1-Methyl Barbituric Acid (200 mg/kg)	173.0 ± 16.4	231.8 ± 41.5 ^b	271.5 ± 61.8 ^b		222.5 ± 36.3	
Control	162.0 ± 9.9	230.0 ± 21.5 ^b	244.5 ± 22.9 ^b	246.0 ± 25.3 ^b		210.0 ± 16.7 ^b
1,3-Dimethyl Barbituric Acid (50 mg/kg)	163.8 ± 13.7	226.0 ± 14.5 ^b	237.8 ± 41.2 ^b	233.3 ± 34.8 ^b		188.3 ± 27.2
1,3-Dimethyl Barbituric Acid (100 mg/kg)	181.3 ± 6.1	229.0 ± 11.4 ^b	240.3 ± 11.1 ^b	250.7 ± 6.9 ^b		193.0 ± 7.0
1,3-Dimethyl Barbituric Acid (200 mg/kg)	146.8 ± 5.7	207.8 ± 12.9 ^b	265.3 ± 29.7 ^b	251.0 ± 29.8 ^b		187.0 ± 11.5 ^b
Control	176.0 ± 10.2	273.0 ± 3.9 ^b	279.5 ± 6.6 ^b		211.5 ± 10.1 ^b	
2-Thiobarbituric Acid (100 mg/kg)	168.3 ± 4.8	262.3 ± 11.0 ^b	246.8 ± 17.3 ^b		208.3 ± 8.8 ^b	
2-Thiobarbituric Acid (200 mg/kg)	140.5 ± 4.8	326.5 ± 25.7 ^c	318.5 ± 32.3 ^b		218.0 ± 20.7 ^b	

^a Serum glucose concentrations were determined on repeated samples from groups of four free-feeding mice at various times after treatment.

^b Significantly greater ($p < 0.05$) than pretreatment value (i.e., 0 min).

^c Significantly greater ($p < 0.05$) than pretreatment value and control at same time interval.

TABLE II. EFFECT OF BARBITURIC ACID ANALOGUES ON GLUCOSE TOLERANCE IN MICE^a.

Treatment	Serum glucose concentration (mg/100 ml ± SEM) Time (min)							
	-30	0	15	30	45	60	90	180
Control (vehicle, pH 2.4)	159.8 ±7.6	193.5 ±13.3	528.0 ^b ±58.4	427.5 ^b ±82.5		390.5 ^b ±88.5	352.8 ^b ±85.4	255.5 ±56.4
Barbituric Acid (200 mg/kg)	142.5 ±11.1	220.8 ±10.5	698.0 ^c ±12.9	609.5 ^c ±18.1		498.5 ^c ±44.5	420.0 ^b ±59.3	270.8 ^b ±58.1
2-Thiobarbituric Acid (200 mg/kg)	146.8 ±5.6	221.5 ±19.4	675.0 ^c ±26.5	525.3 ^c ±54.0		360.5 ^b ±55.7	280.8 ^b ±52.2	160.8 ±10.4
1-Methyl Barbituric Acid (200 mg/kg)	150.3 ±1.8	239.8 ±29.8	691.5 ^c ±29.9	548.5 ^c ±53.6		424.8 ^b ±75.0	321.8 ^b ±58.0	184.3 ±13.6
Control (Vehicle, pH 2.4)	142.6 ±11.4	156.8 ±5.3	540.2 ^b ±20.1		243.0 ^b ±45.5			127.8 ±10.5
1,3-Dimethyl Barbituric Acid (200 mg/kg)	132.0 ±9.3	182.8 ±11.3	590.2 ^b ±49.1		361.5 ^c ±72.3			140.8 ±10.0

^a Serum glucose concentrations were determined on repeated samples from groups of either four (Control, 2-Thio BA, 1-Methyl BA, and BA) or five (Control and 1,3-Dimethyl BA treatments) free-feeding mice. Values at -30 min represent samples obtained immediately before treatment, and values at 0 min represent samples obtained immediately before D-glucose (2.0 g/kg, ip) administration. Time intervals are relative to D-glucose injection.

^b Significantly greater ($p < 0.05$) than pretreatment value (i.e., -30 min).

^c Significantly greater ($p < 0.05$) than pretreatment value and control at same time interval.

TABLE III. EFFECT OF BARBITURIC ACID ANALOGUES ON GLUCOSE TOLERANCE IN ADRENALECTOMIZED MICE^a.

Treatment	Serum glucose concentration (mg/100 ml \pm SEM) Time (min)				
	-30	0	15	45	180
Control (Vehicle, pH 2.4)	166.4 \pm 12.8	174.6 \pm 16.7	451.0 \pm 42.5 ^b	326.6 \pm 52.3 ^b	129.3 \pm 20.6
Barbituric Acid (200 mg/kg)	167.0 \pm 24.6	189.3 \pm 44.2	581.0 \pm 47.5 ^c	538.3 \pm 16.7 ^c	252.0 \pm 8.1 ^c
2-Thiobarbituric Acid (200 mg/kg)	138.2 \pm 10.3	128.0 \pm 15.7	483.6 \pm 37.3 ^b	227.2 \pm 18.4 ^b	81.0 \pm 14.5
1-Methyl Barbituric Acid (200 mg/kg)	158.0 \pm 12.1	171.4 \pm 11.9	534.2 \pm 16.0 ^c	243.3 \pm 23.9 ^b	100.2 \pm 12.0
1,3-Dimethyl Barbituric Acid (200 mg/kg)	147.2 \pm 12.3	135.4 \pm 18.5	509.0 \pm 44.3 ^b	340.5 \pm 57.5 ^b	67.6 \pm 19.2

^a Serum glucose concentrations were determined on repeated samples from groups of five free-feeding mice which had been adrenalectomized 66 hr earlier and maintained on laboratory feed and 0.9% NaCl drinking fluid. Values at -30 min represent samples obtained immediately before treatment, and values at 0 min represent samples obtained immediately before D-glucose (2.0 g/kg, ip) administration. Time intervals are relative to D-glucose injection.

^b Significantly greater ($p < 0.05$) than pretreatment value (i.e., -30 min).

^c Significantly greater ($p < 0.05$) than pretreatment value and control at same time interval.

cose intolerance longer than 15 min after the glucose challenge. In fact, these animals remained intolerant to the sugar for longer than the 180 min duration of the experiment. In contrast, glucose tolerance was not affected at any interval in those adrenalectomized animals receiving either 2-thio BA or 1,3-dimethyl BA. Also, serum glucose concentrations returned to resting levels, or less, in these animals and those pretreated with 1-methyl BA within 180 min after the glucose challenge.

Discussion. Although a dose-dependent hyperglycemic response to BA administration was confirmed in this study, methyl substitution on either one or both of the imino groups of the molecule completely abolished this property of the compound. In addition, this glycemic response to BA was greatly attenuated with the 2-thio substitution. Furthermore, although a slight degree of glucose intolerance was demonstrated by pretreating intact mice with either 2-thio BA or 1-methyl BA, adrenalectomy completely blocked this response to these ureido substituted analogues of BA while pretreatment with 1,3-dimethyl BA elicited no signs of glucose intolerance in either intact or adrenalectomized mice. Therefore, the three derivatives with substitutions on the ureido group (i.e., 2-thio BA, 1-methyl BA, and 1,3-dimethyl BA) demonstrated significantly less activity on these parameters of glycemic homeostasis than the parent BA compound.

The results of this investigation closely parallel the earlier findings of Brückmann and Wertheimer (1) using structure-activity relationship studies on the chemically related compound, alloxan. In comparing BA and alloxan, substitutions on the ureido moiety of either compound greatly compromise the effect each has on glycemic homeostasis. For the alloxan homologues, this means a depressed pancreotoxic potency while with the BA derivatives, a reduced glucose intolerance is observed.

A pharmacologic agent which selectively attenuates inappropriate pancreatic insulin secretions without disturbing other homeostatic systems is desirable in the treatment of clinical hyperinsulinemia as well as several medical afflictions secondary to this metabolic disorder. Although BA administration has been shown to reduce pancreatic insulin secretory activity with no reported direct extra-pancreatic effects, acidic properties and poor lipid solubility are also attributed to this compound. Regardless, the results of this investigation in conjunction with reports from earlier workers (1, 2) indicate the necessity of the unsubstituted ureido moiety in an intact pyrimidine nucleus, as in the BA molecule, to affect the regulation of carbohydrate metabolism. In conclusion, when testing other derivatives of BA which may demonstrate potential utility in the clinical management of hyperinsulinism or related disorders, the unsubstituted ureido moiety must be included

in the structure of the compound being considered.

Summary. Structure-activity relationship studies on the BA molecule were conducted utilizing glycemic responses in mice. Substitutions on the ureido group decreased both the hyperglycemic response and glucose intolerance which BA normally elicits. These results indicate that the unsubstituted ureido moiety is required for the molecule to exert its effects on these parameters of carbohydrate metabolism.

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