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Hypoglycemic Activity of Valeramide, 4-Dimethylamino-N-methyl-2, 2-Diphenyl-, Hydrochloride.* (27319)

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Since the discovery of insulin, numerous chemicals and plant and bacterial extracts have been tested for effectiveness in depressing blood sugar following oral administration. The scope of this search is readily apparent from two recent reviews(1,2). Little success was realized until the orally active hypoglycemic effect of sulfonylureas was rediscovered (3-5). More recently, phenethylbiguanide (DBI) was found to be orally effective in diabetics(6).

Although the sulfonylureas and DBI are effective in some diabetics, their use is primarily in the adult type and the search for compounds with more extensive activity or a different mechanism of action has continued. This report describes studies on the hypoglycemic action of valeramide, 4-dimethylamino-N-methyl-2,2-diphenyl-, hydrochloride (U-6420) (Fig. 1).

Methods. Intact and adrenalectomized rats. These methods, which have been described(8), consist of measuring blood sugars 2 hours after oral administration of the compound in CMC vehicle(9). In an experiment determining influence of U-6420 on hyperglycemic effect of epinephrine or hydrocortisone, male rats were adrenalectomized one week before use. They were maintained on standard laboratory chow and 1% sodium chloride drink, and were fasted overnight before use. Hydrocortisone or epinephrine was given subcutaneously 30 minutes after oral administration of the valeramide. Blood sugars were determined on the Autoanalyzer on blood obtained from the vena cava 2 hours after U-6420 administration.

Eviscerate rats. Male rats (Charles River) weighing 200 ± 5 g were eviscerated by the method of Russell(10) while under anesthesia induced by intraperitoneal injection of cyclopal sodium (32 mg/kg) and subcutaneously injected phenobarbital sodium (132 mg/kg). Following the operation, the animals were connected to a constant infusion pump *via* the jugular vein and received 40 mg glucose/100 g body weight/hour/1.25 ml saline. U-6420 was injected subcutaneously in 0.5 cc CMC at the time glucose infusion was started. Two hours after treatment blood was obtained from the vena cava and analyzed for glucose by the Autoanalyzer. During the experiment animals were kept in a constant temperature box maintained at 26.5 ± 0.5°C.

Blood sugar of alloxan diabetic rats. Alloxan diabetes was produced in fasted intact male rats (Sprague-Dawley) weighing 150-170 g by intravenous injection of 42 mg/kg alloxan monohydrate (Eastman) 2-3 months before use. They were housed in wire metabolism cages and cup fed 10 cc of liquid diet(11) twice a day.

Experimental design was a crossover in which half of the animals served as controls

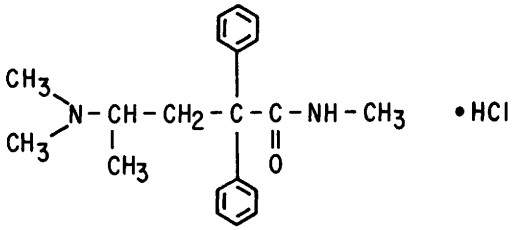

FIG. 1. Structure of valeramide, 4-dimethylamino-N-methyl-2,2-diphenyl-, hydrochloride (U-6420).

* This compound was synthesized and reported to possess oxytoxic and diuretic activity by Moffet *et al.*(7).

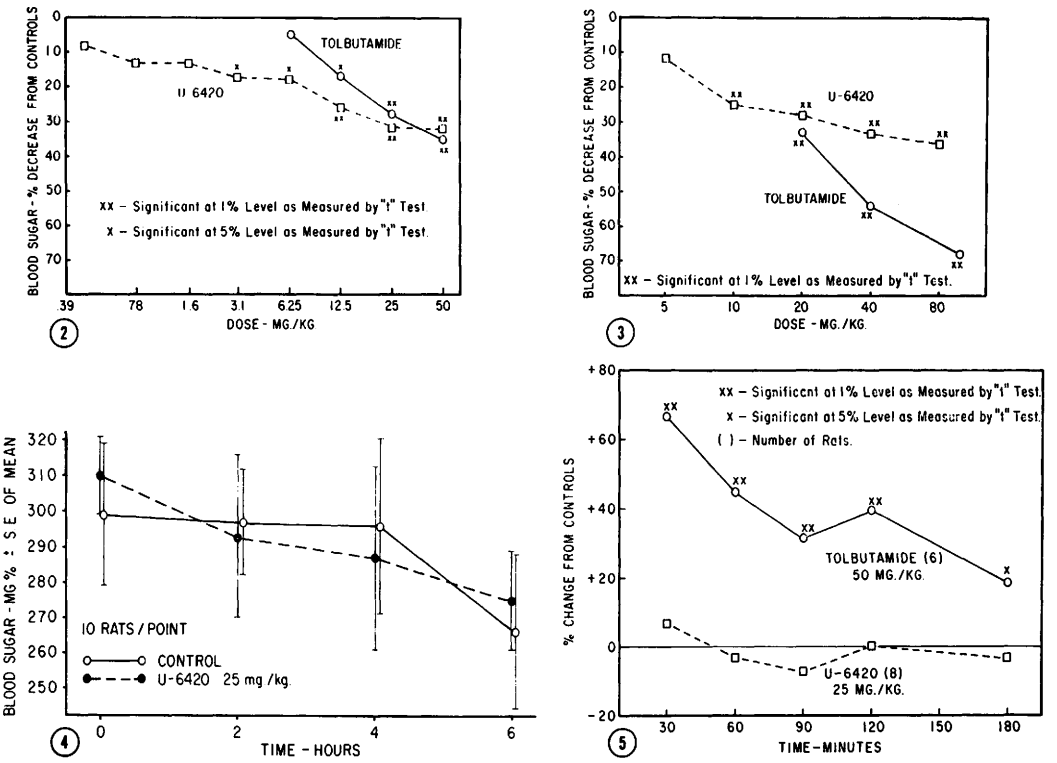

FIG. 2. Comparative hypoglycemic effects of U-6420 and tolbutamide in fasted intact rats 2 hr after oral administration. At least 12 rats/point.

FIG. 3. Comparative effects of U-6420 and tolbutamide on blood sugars of adrenalectomized rats 2 hr after oral treatment. At least 10 rats/point.

FIG. 4. Effects of U-6420 on blood sugars of alloxan diabetic rats. Each animal was used both as control and treated. Blood sugar determined by glucostat. 10 rats/point.

FIG. 5. Effects of U-6420 and tolbutamide on glucose U-C^{14} oxidation by fasted intact rats. Control animals were studied simultaneously with treated rats.

and half were treated on Week 1. One week later those which had been controls were treated and those which had been treated served as controls. Blood was taken from the tail immediately prior to treatment, and 2, 4, and 6 hours later. Sugars were determined by the glucostat method(12).

Glucose U-C^{14} oxidation by intact rats. The method used has been described(13). It consists essentially of I.P. injection of glucose U-C^{14} (5×10^5 CPM) ½ hour after oral administration of the compound. Animals (intact Sprague-Dawley males) were placed in glass metabolic units and expired CO_2 trapped in sodium hydroxide which was sampled periodically, precipitated as $BaCO_3$, and counted in a gas flow counter (Baird Atomic). Values were corrected for self-absorption by use of a calibration curve for $BaCO_3$ for this instrument.

Results. Data from fasted intact rats (Fig 2) were pooled from 3 separate experiments. Since the slopes of the curves were shallow and that of U-6420 was different from that for tolbutamide, an exact potency ratio was difficult to assign. However, based on the minimal effective dose of 3.1 mg/kg for U-6420, and 12.5 mg/kg for tolbutamide, the valeramide was approximately 4 times more active than tolbutamide.

In adrenalectomized rats U-6420 did not produce a good dose response curve (Fig. 3). Consequently, potency estimates as compared to tolbutamide were difficult. One can conclude that U-6420 was active at 10 mg/kg as a hypoglycemic agent in the adrenalectomized rat.

Increased oxidation of glucose U-C^{14} was not observed following U-6420 treatment (Fig. 4). Tolbutamide, as anticipated, sig-

TABLE I. Effects of U-6420 on Blood Sugars of Eviscerate Rats, 2 Hours after Subcutaneous Administration. Glucose infused at 40 mg/100 g body wt/hr.

No. rats	Treatment	Dose, mg/kg	Avg blood sugar 2 hr - 0 hr, mg %
Exp. I			
5	Controls	—	128
2*	U-6420	50	145
Exp. II			
3	Controls	—	123
3	U-6420	25	163

* 3 rats died.

nificantly increased oxidation of glucose. The valeramide was also incapable of lowering blood sugars of alloxan diabetic (Fig. 5) or eviscerate rats (Table I). When epinephrine or hydrocortisone was given to adrenalectomized rats following treatment with U-6420, they both maintained the ability to increase blood sugar (Table II).

Discussion. Valeramide, 4-dimethylamino-N-methyl-2,2-diphenyl-, hydrochloride (U-6420) has been found to exhibit a greater (4×) hypoglycemic effect than tolbutamide in fasted intact rats. Lack of a dose-related response in adrenalectomized rats would suggest that U-6420 acts by inhibiting the effects of adrenal hormones. Data showing that the valeramide did not counteract the hyperglycemic effects of epinephrine or hydrocortisone and that it was effective as a hypoglycemic agent in adrenalectomized rats does not support the conclusion that it acts by inhibition of adrenal hormone secretion or action.

TABLE II. Effects of U-6420 on Epinephrine or Hydrocortisone-Induced Hyperglycemia of Adrenalectomized Rats. U-6420 given ½ hr before epinephrine or hydrocortisone. Blood sugars determined 2 hr after hormone inj.

No. rats	Treatment	Dose	Blood sugar
8	Controls	—	48 ± .93*
8	Epinephrine	15 μg	94 ± 2.38
8	U-6420	50 mg/kg	21 ± 3.90
8	Epinephrine + U-6420	15 μg + 50 mg/kg	80 ± 5.60
6	Controls	—	56 ± 3.47
6	Hydrocortisone	5 mg/kg	74 ± 2.75
6	U-6420	50 mg/kg	39 ± 3.67
6	Hydrocortisone + U-6420	5 mg/kg + 50 mg/kg	55 ± 1.98

* ± stand. error of mean.

Insulin as well as tolbutamide has been shown to increase oxidation of glucose U-C^{14} to $C^{14}O_2$(13). Data reported here support the previous observation that sulfonylureas increase glucose oxidation. Inability of U-6420 to stimulate glucose oxidation indicates that it does not lower blood glucose by an insulin or sulfonylurea-like mechanism. In further support of a non-insulin-like action is data showing a lack of effect on blood sugars of alloxan diabetic or eviscerate rats, since insulin is effective in these animal preparations (14,15). Since DBI has been observed to lower blood sugars of alloxan diabetic and eviscerate animals(16,17), it is concluded that U-6420 does not act *via* a DBI-type mechanism.

The possibility that U-6420 acts by inhibition of liver glucose output seems to be the most acceptable explanation for its mechanism of hypoglycemic effect. Although lack of effect on blood sugar of alloxan diabetics might be used against this hypothesis, it is known that effect of insulin on the liver of the diabetic requires 6-24 hours(18). Therefore, the failure of U-6420 to lower blood sugar of alloxan diabetics does not invalidate a liver mechanism, but means that it does not have a peripheral action.

The present data show that U-6420 is a unique blood sugar-lowering agent. Its mechanism of action is not like that of insulin, tolbutamide, or DBI, and indicates that there are many poorly understood ways to lower blood sugar.

Summary. Valeramide, 4-dimethylamino-N-methyl-2,2-diphenyl-, hydrochloride (U-6420) was found to be about 4 times more potent than tolbutamide in lowering blood sugars of fasted intact rats. Although U-6420 was active in depressing blood sugars of adrenalectomized rats, the response was not dose-related, as was the case with tolbutamide. Since hydrocortisone or epinephrine still increased blood sugars of U-6420-treated adrenalectomized rats, it was concluded that the mechanism of hypoglycemic action was not due to inhibition of the metabolic effects of adrenal hormones. It was also found that

U-6420 did not lower blood sugars of alloxan diabetic or eviscerate rats, nor increase oxidation of glucose U-C^{14} by fasted intact rats. It was concluded from these studies that the mechanism of action of U-6420 was not like that of the sulfonylureas, biguanides, or insulin. The most probable mechanism was that of decreasing glucose output by the liver.

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Effect of Dietary Fats and Vitamin E on Oxidative Denaturation of Serum Low Density Lipoproteins.* (27320)

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We have previously shown that low density lipoproteins isolated from human or chick serum by ultracentrifugation can be denatured *in vitro* by exposure to the hydroperoxides of methyl linoleate(1). Furthermore, the hydroperoxide of methyl linoleate selectively or preferentially denatured β-lipoproteins and not other serum lipoproteins or protein constituents. It has recently been shown that low density lipoproteins contain a major share of the total serum tocopherol (2). In a deficiency of antioxidants or synergists therefore a diet rich in unsaturated fatty acids may cause serum low density lipoproteins to be susceptible to oxidative denaturation. In the present study, the stability or lability of low density lipoproteins of the Sf 0-20 class obtained from sera of chicks fed corn oil or hydrogenated coconut oil and with or without DL-α-tocopherol was compared in the presence of a catalytic amount of hemin.

Method. Day-old female chicks (New Hampshire Columbian Cross) were divided into 5 groups of 35 chicks each and kept on the experimental diets(3) for 2 weeks. These diets contained 2 or 10% stripped corn oil† or 10% hydrogenated coconut oil with and without 8 mg % of DL-α-tocopherol.‡ The birds were bled via heart puncture, serum samples from birds in each group pooled and the fraction composed of chylomicrons and low density lipoproteins of Sf 20-400 removed by an initial centrifugation. Low density lipoproteins of Sf 0-20 class were isolated(4),

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‡ DL-α-tocopherol was furnished through courtesy of Merck & Co., Inc., Rahway, N. J.