Pharmacologic Studies with 3-(Phenylpropoxy)guanidine Cyclohexanesulfamate

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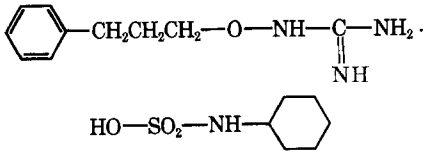
Some comparisons of the pharmacologic properties of 3-(phenylpropoxy)guanidine cyclohexanesulfamate (U-16,178F) and dextroamphetamine sulfate are reported. While less potent on a milligram basis than dextroamphetamine sulfate, U-16,178F is an effective anorexigenic agent in dogs, rats, and mice at nontoxic doses. In contrast to dextroamphetamine sulfate, U-16,178F, after a single intraperitoneal dose, does not increase motor activity in mice. However, when administered chronically, by admixing it in the diet, U-16,178F causes an increase in motor activity after 24 hr. on diet. U-16,178F, in contrast to amphetamine, causes an initial fall in blood pressure following intravenous administration in the anesthetized dog. It is about one-tenth as potent as dextroamphetamine sulfate in lowering brain catecholamine levels and, unlike dextroamphetamine sulfate, has no tryptamine potentiating activity but protects against maximal electroshock. It has much less antireserpine activity than dextroamphetamine sulfate. U-16,178F also differs from dextroamphetamine sulfate in that changes in ambient temperature have little effect on its acute toxicity.

THE CHEMISTRY and preliminary pharmacology of a series of aralkoxyguanidines was previously reported by Martin *et al.* (1). From these studies 3-(phenylpropoxy)guanidine nitrate was selected as a potential anorexigenic agent. However, the potential hazards involved in preparing and purifying larger quantities of the nitrate salt for further studies prompted the substitution of the cyclohexanesulfamate salt for the nitrate. The cyclohexanesulfamate salt was preferred over the hydrochloride since the latter compound retained a slight odor even after several recrystallizations. Additional pharmacologic studies and comparisons with dextroamphetamine sulfate have been completed and are the subject of this report. U-16,178F has the following chemical structure:



METHODS

A comparison was made of the acute effects of U-16,178F and dextroamphetamine sulfate on food intake in a group of 32 adult mongrel dogs. A crossover design for an 8-point assay was used (2). The dogs were housed in individual cages in a relatively constant-temperature (75° F.) dog room and were fed daily at the same hour. Dog pellets (Purina laboratory chow) were presented for a 30-min. period and the amount eaten was recorded. Drinking water was available at all times. When drugs were tested, they were given orally in a gelatin capsule 1 hr. before presentation of food. The dogs employed had been on this feeding schedule for at least 3 weeks before the assay started. Drug effect was measured as the per cent decrease in food intake for each dog. Control intake was the average of the 5 control days preceding the assay and the 3 control days between the crossover.

To obtain additional information concerning the influence of U-16,178F on food intake and body weight in the dog, it was also administered on a chronic basis. For comparison, dextroamphetamine sulfate was tested under the same conditions, although not simultaneously. Two groups of four mature mongrel dogs were used. These dogs were housed in individual cages in a relatively constant-temperature (75° F.) dog room. Food was available to the dogs from 8:00 a.m. until 3:00 p.m. daily with water available continuously. The dogs used had been on this feeding schedule for at least 1 month. Food consumption was measured daily. Body weight was determined before and after drug treatment. Drugs were administered orally (gelatin capsule) twice daily at 7:00 and 11:00 a.m. for 5 days. Drug effect was assessed as the per cent decrease in food intake for each dog and the decrease in body weight from the beginning to the end of the treatment period. Control food intake was the average of the 5 to 7 control days preceding the treatment period.

Anorexigenic activity was also determined in rats. Upjohn Wistar male rats weighing 190 to 210 Gm. were housed individually and fasted 22 hr. Six rats were used at each dose level. U-16,178F was tested at 3, 10, and 30 mg./Kg. and dextroamphetamine sulfate at 0.3, 1, and 3 mg./Kg. The drugs, dissolved in aqueous methylcellulose (0.25%) so that the dose was contained in a volume of 1 ml./100 Gm. body weight, were administered by oral intubation. Thirty minutes after dosing, a stock ground diet (Upjohn BA) was supplied and the animals were allowed to feed *ad libitum* for a 2-hr. period. The amount of food each rat consumed was recorded and the potency of U-16,178F relative to dextroamphetamine sulfate determined by means of a parallel line assay.

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TABLE I—EFFECT OF ACUTE SINGLE DOSE ADMINISTRATION 1 hr. BEFORE ON FOOD INTAKE IN DOGS

Compd.	Dose, mg./Kg.	% Inhib.	Compd.	Dose, mg./Kg.	% Inhib.
U-16,178F	1.25	59	Dextroamphetamine sulfate	0.0625	45
U-16,178F	2.5	55	Dextroamphetamine sulfate	0.125	80
U-16,178F	5.0	73	Dextroamphetamine sulfate	0.25	87
U-16,178F	10.0	75	Dextroamphetamine sulfate	0.5	93

The effects of U-16,178F and dextroamphetamine sulfate on food intake, body weight, and motor activity were determined in mice. Groups of eight Carworth Farm male mice (22–24 Gm.) were weighed and then housed together and fed a stock ground diet (Upjohn BA) containing various concentrations of the test compound. The control group received ground diet only. After 24 hr. on this diet, food consumption and body weight changes were determined. Motor activity was measured in actophotometers[1] after the method of Dews (4). After a 10-min. acclimation period in the actophotometers, motor activity of individual mice was recorded for 1 hr. An 8 × 8 Latin square design balanced with respect to treatments, actophotometers, and time of day was used. The mice were left on test diet until they were placed in the actophotometers. The dose of drug ingested by each group of mice was calculated.

Motor activity effects of U-16,178F and dextroamphetamine sulfate were also compared when the compounds were administered intraperitoneally to groups of eight mice. Immediately after injection, the mice were placed in individual actophotometers, allowed to acclimate for a 10-min. period, and then motor activity was recorded for 1 hr. The same 8 × 8 Latin square as described above was used.

To study the effects of U-16,178F and dextroamphetamine sulfate on the overt behavior of cats, groups of eight mature cats were placed together in a small room. Their behavior was evaluated subjectively using a modification of the rating scale devised by Norton (5). This rating system consists of 35 evaluations of cat behavior grouped in six categories: stimulation, sociability, defensive hostility, aggressive hostility, contentment, and a miscellaneous group. The behavior of the cats was scored 7 times at hourly intervals. After the second rating each cat received a dose of one of the drugs, administered orally in a gelatin capsule. Each trial included all dose levels of each drug plus a lactose control. All dose levels of each drug were tested 6 times with the doses randomized among the 16 cats used. No animals were used less than twice or more than 4 times. In all but the first trial, the observer was unaware of the dosing schedule used.

Blood pressure effects of intravenous doses of U-16,178F were determined in 10–14-Kg. mongrel dogs. The dogs were anesthetized with sodium thiopental, 20 mg./Kg. intravenously, followed by sodium barbital, 250 mg./Kg. intravenously. The trachea of each dog was cannulated to ensure an adequate airway, and a femoral artery was cannulated to record blood pressure. Pressure tracings were obtained on a Grass polygraph through a Statham P-23AC transducer. Injections were made into an exposed femoral vein. Responses to epinephrine, acetylcholine, and histamine standards were determined before and after administration of U-16,178F. In two of the four dogs, a 1-min. period of bilateral carotid artery occlusion was added as a test for sympathetic reactivity.

Brain norepinephrine and serotonin levels were determined in the mouse 2 hr. after intraperitoneal administration of U-16,178F and dextroamphetamine sulfate. The method used for extraction of norepinephrine and serotonin from the homogenates was essentially that of Shore and Olin (6) as utilized by Mead and Finger (7). Norepinephrine was determined by the method of Shore and Olin (6) and serotonin was assayed using a modification of the method of Snyder, Axelrod, and Zweig (8).

Tryptamine potentiation, reserpine antagonism, and maximal electroshock protecting activities of U-16,178F and dextroamphetamine sulfate were determined using groups of six Carworth Farm male mice weighing 18–22 Gm. U-16,178F and dextroamphetamine sulfate were administered intraperitoneally in aqueous methylcellulose (25%) at multiple dose levels distributed at 0.3 log intervals. In the tryptamine potentiation test the mice were pretreated with U-16,178F or dextroamphetamine sulfate. Thirty minutes later they were challenged with 25 mg./Kg. of tryptamine hydrochloride intravenously and observed for tryptamine-like symptoms. The 25-mg./Kg. dose of tryptamine hydrochloride does not produce symptoms in control mice while animals treated with an effective dose (*e.g.*, 100 mg./Kg. i.v.) show the following symptoms: hind leg spread, arched back, pawing, head weave, and tremors (9).

In the reserpine antagonism tests one group of mice was treated with 2.5 mg./Kg. of reserpine intraperitoneally and 2 hr. later challenged with the test compound intraperitoneally. Thirty minutes later the degree of ptosis was noted using the 4-point scale of Rubin (10). Another group of mice was treated with the test compound intraperitoneally and after 30 min. challenged with 2.5 mg./Kg. of reserpine intraperitoneally. In each situation control reserpine groups were tested simultaneously. All mice receiving only reserpine had ptosis scores of 4 (complete closure). In the electroshock test mice were treated with the test compounds and 1 hr. later were shocked *via* ear clip electrodes with a 60 c. current for 0.2 sec. at a current intensity of 25 ma. (11).

The influence of ambient temperature on the acute toxicity of U-16,178F and dextroamphetamine sulfate was determined in mice. Groups of six Carworth Farm male mice weighing 18–22 Gm. were injected either intraperitoneally or orally with the compound dissolved in aqueous methylcellulose (0.25%). After injection, the mice were placed in mouse cages[2] that had been divided in half by a plastic wall making compartments 28 × 18 × 14

[1] Metro Industries, Mineola, L. I., N. Y.

[2] Keystone Plastics Co., Media, Pa.

TABLE II—EFFECTS OF CHRONIC DOSING ON FOOD INTAKE AND BODY WEIGHT IN DOGS

	U-16,178F	Dextroamphetamine Sulfate
Dose, mg./Kg. b.i.d.	5.0	0.5
Av. % inhibition of food intake ± S.E.	68.3 ± 4.0	83.8 ± 6.9
Av. wt. loss in Kg. ± S.E.	1.25 ± 0.4	0.98 ± 0.06

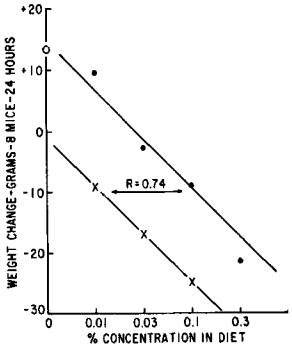

Fig. 1—Parallel bioassay on weight change in mice. U-16,178F (●) vs. dextroamphetamine sulfate (×); control (○).

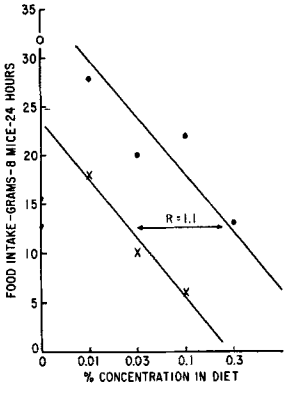

Fig. 2—Parallel bioassay on inhibition of food consumption in mice. U-16,178F (●) vs. dextroamphetamine sulfate (×); control (○).

cm. Standard perforated metal tops were used. Cages with one group of mice at each dose level were placed in a cabinet (120 × 54 × 56 cm.) under the laboratory bench at 22° C., similar groups were placed in a constant-temperature oven (74 × 46 × 46 cm.) at 34° C., and in a refrigerator at 4° C. The two compounds were run in parallel. The high dose used for the oral test with U-16,178F was 400 mg./Kg. and the high dose for the intraperitoneal test for both U-16,178F and dextroamphetamine sulfate was 200 mg./Kg. Decreasing at 0.3 log intervals, the lowest dose of U-16,178F both orally and intraperitoneally was 25 mg./Kg. and the lowest dose of dextroamphetamine sulfate administered was 1.56 mg./Kg. Although the mice were examined at 15-min. intervals for 2 hr., the LD_{50}'s were computed by the method of Spearman and Karber (see *Reference 3*) for the 1 and 2-hr. period.

TABLE III—EFFECT ON MOTOR ACTIVITY OF U-16,178F AND DEXTROAMPHETAMINE SULFATE WHEN ADMIXED WITH DIET

Compd.	Dose Ingested, mg./Kg.	Total Counts 8 Mice for 60 min.
Control	...	4,883
U-16,178F	478	8,437
U-16,178F	251	8,301
U-16,178F	66	7,121
U-16,178F	29	5,975
Dextroamphetamine sulfate	77	11,024
Dextroamphetamine sulfate	36	9,202
Dextroamphetamine sulfate	20	8,681

RESULTS

Acute Effects on Food Intake in Dogs—The results of the comparison of U-16,178F and dextroamphetamine sulfate are shown in Table I. The potency of U-16,178F relative to dextroamphetamine sulfate in this test on a mg./Kg. basis was 0.025 with 95% confidence limits of 0.002–0.078. In this analysis the dog to dog standard deviation for per cent inhibition of food intake was 37%. The dose response slope for U-16,178F was 25%/tenfold increase, while that of dextroamphetamine sulfate was 50%/tenfold increase, but this difference was not statistically significant.

Chronic Effects on Food Intake and Body Weight in Dogs—U-16,178F was tested at 5.0 mg./Kg. b.i.d. and dextroamphetamine sulfate at 0.5 mg./Kg. b.i.d. for 5 days. Body weight loss with the two compounds was about the same, while dextroamphetamine sulfate caused a somewhat greater inhibition of food intake (Table II).

Anorexigenic Activity in Rats—Analysis of the food intake data obtained in rats treated with U-16,178F and dextroamphetamine sulfate showed that on a mg./Kg. basis the potency of U-16,178F relative to dextroamphetamine sulfate was 0.030 with 95% confidence limits of 0.007–0.058.

Effects of Body Weight Change, Food Intake, and Motor Activity in Mice When Admixed with Diet—U-16,178F was tested at 0.01, 0.03, 0.1, and 0.3% concentrations in the diet, while dextroamphetamine sulfate was tested at 0.01, 0.03, and 0.1% concentrations. Effects on body weight change and on food intake are shown in Figs. 1 and 2. On a mg./Kg. basis, U-16,178F was about $^1/_5$ as potent as dextroamphetamine sulfate in causing body weight loss and $^1/_{12}$ as potent in inhibiting food intake in this procedure.

Effects on motor activity after the mice had been on the diet containing the test compound for 24 hr. are shown in Table III. Analysis of variance of motor activity data showed no significant difference between the control and the mice on the diet containing U-16,178F, while the motor activity of the mice on the diet containing dextroamphetamine sulfate was significantly higher than that of the controls. However, when covariance analysis was used to compare motor activity at doses which caused a comparable inhibition of food intake or weight loss, it was found that the motor stimulant effects of U-16,178F were somewhat less than those of dextroamphetamine sulfate, but the difference was not statistically significant.

TABLE IV—EFFECT ON MOTOR ACTIVITY OF U-16,178F AND DEXTROAMPHETAMINE SULFATE WHEN ADMINISTERED 10 min. BEFORE BY INTRAPERITONEAL INJECTION

Compd.	Dose, mg./Kg.	Total Counts 8 Mice for 60 min.
Control	...	6,863
U-16,178F	40	6,616
U-16,178F	20	5,309
U-16,178F	10	6,206
U-16,178F	5	5,763
Dextroamphetamine sulfate	5	18,138
Dextroamphetamine sulfate	2.5	16,690
Dextroamphetamine sulfate	1.2	8,918

Motor Activity in Mice After Intraperitoneal Administration—The effects of U-16,178F and dextroamphetamine sulfate on motor activity in mice after intraperitoneal administration are summarized in Table IV. Analysis of variance of these data showed that while dextroamphetamine sulfate at the 5 and 2.5 mg./Kg. doses caused a statistically significant increase in motor activity compared to controls, U-16,178F had no effect on motor activity at any of the doses tested.

Effect on Overt Behavior in Cats—U-16,178F was administered orally at 1, 3, 10, and 30 mg./Kg. and dextroamphetamine sulfate at 1, 3, and 10 mg./Kg. Both U-16,178F and dextroamphetamine sulfate increased the incidence of head turning, head bob, piloerection, and dilated pupils. However, U-16,178F was only $^1/_{10}$ to $^1/_{30}$ as potent as dextroamphetamine sulfate. Both compounds decreased overt signs interpreted as contentment while sociability was reduced only by dextroamphetamine sulfate. Measures of hostility were not increased by either compound, while spontaneous motor activity was decreased by both. Emesis, diarrhea, hallucinations, and poor contact were not observed in any of the animals, although a slight increase in the incidence of salivation was noted with U-16,178F. A time course evaluation of the data indicated that the maximum activity of both compounds was seen during the first and second hourly observations with a gradual decrease in activities thereafter.

Effects on Blood Pressure, Autonomic Standards, and Carotid Occlusion Response in Anesthetized Dogs—U-16,178F was tested intravenously in four dogs. Doses of 0.5, 1.0, 2.0, 8.0, and 16.0 mg./Kg. were employed in the first pair of animals and 4.0, 8.0, and 16.0 mg./Kg. in the second pair. It produced a brief dose-related fall in arterial pressure reaching −70 mm. Hg for 3 min. at 16 mg./Kg. The hypotensive response to U-16,178F was not blocked by a dose of propranolol (1 mg./Kg.) which completely blocked the depressor action of 10 mcg. of isoproterenol. This would indicate that the hypotensive effect of U-16,178F is not the result of β-adrenergic stimulation. The blood pressure response to carotid occlusion was unchanged at the lower doses of U-16,178F; however, following the 16-mg./Kg. dose, the blood pressure response to carotid occlusion was depressed. Responses to histamine and acetylcholine standards were not changed in any of the four dogs. The pressor responses to epinephrine after all doses of U-16,178F were increased; however, the effect was small (average 11% greater) and was not dose related. The duration of the epinephrine induced pressor response was also somewhat increased (17%–54% longer) and the effect was directly related to the U-16,178F dose.

Catecholamine Depleting Activity—The effects of U-16,178F and dextroamphetamine sulfate on brain catecholamine levels in mice are summarized in Table V. These results show that the norepinephrine values were slightly decreased at each dosage level of U-16,178F with the effect of the 100 mg./Kg. dose approximating the decrease produced by 10 mg./Kg. of dextroamphetamine sulfate. Neither U-16,178F nor dextroamphetamine sulfate altered the serotonin levels.

Effects Against Tryptamine, Resperine, and Electroshock—U16,178F at doses up to 100 mg./Kg. caused no potentiation of tryptamine activity in the mouse while the ED_{50} of dextroamphetamine sulfate for this activity was 18 mg./Kg. U-16,178F

TABLE V—EFFECT OF U-16,178F AND DEXTROAMPHETAMINE SULFATE ON BRAIN AMINE LEVELS IN MICE

Compd.	Dose, mg./Kg.	Norepinephrine: Determinations, No.	Norepinephrine: Concn., mcg./Gm. ± S.E.M.	Norepinephrine: % of Control	Serotonin: Determinations, No.	Serotonin: Concn., mcg./Gm. ± S.E.M.	Serotonin: % of Control
Saline	...	14	0.53 ± 0.06	100	6	0.94 ± 0.05	100
U-16,178F	10	8	0.46 ± 0.05	87	7	0.88 ± 0.08	94
U-16,178F	30	8	0.44 ± 0.05	83	8	0.86 ± 0.05	93
U-16,178F	100	8	0.43 ± 0.06	81	8	0.96 ± 0.06	102
Dextroamphetamine sulfate	1	4	0.55 ± 0.05	103	4	0.86 ± 0.03	92
Dextroamphetamine sulfate	3	6	0.52 ± 0.06	98	6	0.87 ± 0.07	93
Dextroamphetamine sulfate	10	8	0.42 ± 0.04	79	8	0.96 ± 0.06	102

TABLE VI—EFFECTS OF AMBIENT TEMPERATURE ON ACUTE TOXICITY IN MICE

Temp., °C.	LD_{50}, mg./Kg.: Dextroamphetamine Sulfate (i.p.) 1 hr.	Dextroamphetamine Sulfate (i.p.) 2 hr.	U-16,178F (i.p.) 1 hr.	U-16,178F (i.p.) 2 hr.	U-16,178F (p.o.) 1 hr.	U-16,178F (p.o.) 2 hr.
34°	4.5	4.5	79	79	284	224
22°	>12.5	5.6	126	100	400	284
4°	142.0	142.0	142	142	400	356

showed weak antireserpine activity at 100 mg./Kg. On a mg./Kg. basis, U-16,178F was about $^1/_{50}$ as active as dextroamphetamine sulfate when given after reserpine and $^1/_{16}$ as active when given before reserpine. U-16,178F had an ED_{50} of 89 mg./Kg. against maximal electroshock while dextroamphetamine sulfate at doses up to 50 mg./Kg. gave no protection.

Ambient Temperature on Acute Toxicity in Mice—Variations in temperature caused a significant change in the toxicity of dextroamphetamine sulfate but had little influence on the toxicity of U-16,178F. Comparing the LD_{50}'s at 4° and 34° C. there was a thirtyfold change in toxicity with dextroamphetamine sulfate while U-16,178F had less than a twofold change. The results of this experiment are summarized in Table VI.

DISCUSSION

U-16,178F is structurally different from the present marketed anorexigenic agents. As originally pointed out by Modell (12) all of the available anorexigenic agents to date have a phenethylamine moiety in the molecule. This moiety is not present in U-16,178F.

U-16,178F is an effective anorexigenic agent in dogs, rats, and mice. This activity is considered to be due to its central appetite depressant effect and not due to nauseant or anticholinergic activity. No emesis was observed with U-16,178F in dogs or cats at any of the doses tested, and no anticholinergic activity was seen when it was tested against blood pressure effects of intravenous acetylcholine in the anesthetized dog.

When the acute and chronic effects of U-16,178F on anorexigenic activity in the dog are compared, the data suggest that U-16,178F is more effective when given repeatedly than when given in a single dose. This might indicate that U-16,178F has a slower onset of effect than does dextroamphetamine sulfate and/or that there is a less rapid development of tolerance to U-16,178F than to dextroamphetamine sulfate.

A difference between the acute and chronic effects of U-16,178F was demonstrated in the motor activity measurements in mice. An intraperitoneal dose of U-16,178F caused no increase in motor activity when measured for a 1-hr. period 10 min. after administration. However, when administered chronically by admixing it in the diet, it caused an increase in motor activity after 24 hr. on the diet. In contrast, dextroamphetamine sulfate causes an increase in motor activity when given acutely or chronically.

When given intravenously in the anesthetized dogs, U-16,178F produced a brief fall in arterial pressure, followed by a modest rise while the amphetamine is known to cause a definite pressor effect as previously reported (13). The hypotensive effect of U-16,178F was not blocked by propranolol. The secondary rise in pressure seen with U-16,178F coupled with the observation that the blood pressure response to carotid occlusion was depressed following the 16 mg./Kg. dose suggests that U-16,178F has some propensity for releasing norepinephrine and lowering catecholamine stores. This is in agreement with the finding that U-16,178F causes some decrease in brain norepinephrine.

Other qualitative and quantitative differences between U-16,178F and dextroamphetamine sulfate suggest that U-16,178F may be a different type of anorexigenic agent. It is about $^1/_{10}$ as potent as dextroamphetamine sulfate in depleting brain catecholamine levels. Unlike dextroamphetamine sulfate, it had no tryptamine potentiating activity and has much less antireserpine activity. U-16,178F gave protection against maximal electroshock while dextroamphetamine sulfate did not exhibit this activity. The toxicity of U-16,178F was not appreciably increased when the LD_{50} was determined at 30° C. instead of 4° C., while the LD_{50} of dextroamphetaine sulfate increased thirtyfold in this situation.

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