A RAPID METHOD FOR ASSESSING DRUG INHIBITION OF FEEDING BEHAVIOR[1]

ADOLPH P. ROSZKOWSKI AND NANCY M. KELLEY[2]

Biological Research Department, Research Division, McNeil Laboratories, Inc., Fort Washington, Pennsylvania

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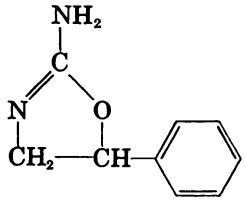
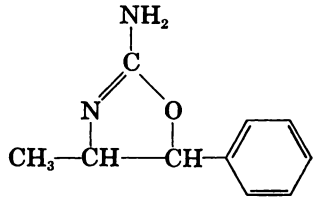
The early experimental findings of Ehrich and Krumbhaar (1937) in rats and those of Alpern *et al.* (1941) in dogs indicated that ingestion of amphetamine caused a depression of food consumption. Similar findings in humans were observed by a number of clinical investigators (Davidoff and Reifenstein, 1937; Nathanson, 1937; Ulrich, 1937) and soon thereafter other investigators indicated that amphetamine could advantageously be used as an appetite depressant in the clinical management of obesity (Lesses and Myerson, 1938; Rosenberg, 1939).



2-Amino-5-phenyloxazoline

McN-742



2-Amino-4-methyl-5-phenyloxazoline

McN-822

Such studies prompted pharmacologists to devise experimental procedures which could be used to demonstrate drug-induced anorexia. Among the first investigators to attempt such an experiment was Tainter (1944).

Most of the procedures employed to demonstrate anorexia consequent to drug action proved to be difficult to carry out in that, frequently, the drug had to be administered over extended time intervals; quantification of activity was then unreliable.

The present investigation was undertaken with a view toward rectifying these difficulties. Accordingly, an attempt was made to devise a procedure which would be rapid, simple, and highly quantitative with respect to appraisal of anorectic activity.

In the course of evaluating this procedure by tests with various anorexigens, two agents which were synthesized and described chemically by Poos *et al.* (1963) appeared to display striking activity as feeding suppressants. The structures of these substances are:

A description of the relative anorectic properties of these agents and other well-known anorexigens will be presented.

METHODS. *Assessment of drug-induced anorexia.* Male Sherman albino rats and, subsequently, Holtzman rats weighing between 250 and 658 grams were used. The animals were housed individually in cages placed in a room maintained at approximately 22°C. Usually an experiment was carried out with a full rack of 60 animals.

The rats were fed a standard rat pellet diet (Rockland) and water *ad libitum*. In addition, it was found that the rats were extremely fond of beef broth (0.75% aqueous solution of Bacto-beef broth available as a bacteriological culture medium in 0.9% NaCl) and would consume this material in the presence of and in preference to rat food.

The broth was presented in a centrifuge tube

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[2] Present address: Predoctoral cardiovascular trainee (Grant #HTS5362, N. H. I., USPHS) Department of Pharmacology, Temple University Medical School, Philadelphia, Pennsylvania.

graduated to 50 ml in 1-ml intervals. The tubes were inverted and clamped to a standard cage, with a stainless steel sipper tube inserted in a rubber stopper. The quantity of broth was measured directly from the graduated scale upon placement on the cage. Control broth consumption was noted for 4 days; on the fifth day the animals were randomized, weighed, and divided into groups varying in number from 7 to 11, usually 10.

The animals were used to test drug activity at intervals of no less than 7 days. One hour prior to feeding, the rats were treated with varying amounts of drug, given orally by stomach tube in aqueous solution or suspension, the volume of which was kept constant according to the weight of the animal. A control group was given an equivalent volume of water orally. After termination of the 15-minute broth consumption "test" period, the tubes were removed from the cages. The amount of broth consumed was expressed in ml/kg; mean broth consumption was determined for the control group and the drug-treated groups. Using the analysis of variance procedure (Snedecor, 1956), the means of the various drug-treated groups were compared with the mean of the control group to determine if any statistical difference in broth consumption occurred.

It was found that drug activity could also be assessed in a quantal manner by obtaining percentages of animals which consumed at least 5 ml/kg of broth as opposed to those which did not. This was possible since control experiments revealed that all control animals at all times consumed more than 5 ml/kg of broth. Regular dose-response relationships were observed when different dose levels of the same compound were administered to different groups. Then, by use of conventional probit transformation procedures as described by Litchfield and Wilcoxon (1949), ED50 determinations were calculated for a drug's ability to suppress feeding.

Appraisal of drug-evoked changes in behavior and locomotor activity. Male rats of the same derivation as those used in the anorectic experiments were studied after oral administration of drugs at various dose levels, to appraise drug-induced gross behavioral changes which would incapacitate the animal, such as tremor, convulsion, or immobilization due to apparent depression.

Using rats of the same type, drug-induced changes in locomotor activity were also measured by the number of revolutions produced by a rat when placed in a roller, treadmill (Wahmann) cage. Cross-over type experiments were carried out using 6 rats and 6 cages in each study. In the first study the rats were numbered 1 to 6, placed in cages numbered 1 to 6 and allowed to revolve the cages for exactly 2 hours. The number of revolutions, which were counted mechanically, was recorded. Following this interval the rats were given orally the following substances in numerical order: 1) H_2O, 2) methamphetamine, 3) *d*-amphetamine, 4) phenmetrazine[3], 5) McN-742, and 6) McN-822. These drugs were given at predetermined ED50 anorectic dose levels. Fifteen minutes after the termination of the first "revolving" period the rats were again placed in their respective cages for another 2-hour period with a recording of the revolutions made. After a 3- or 4-day period without drug treatment the rats were again used in the same manner but rotated so that rat #1 was given drug #2, methamphetamine, instead of water, and rat #2 received drug #3, *etc.* Such a rotation was repeated at 3-day intervals until each animal was subjected to each treatment twice.

In a second study, using the same method, the following substances were used: 1) H_2O, 2) methamphetamine, 3) amphetamine, 4) *d*-amphetamine, 5) phenmetrazine, and 6) diethylpropion.[4]

The activity for each drug treatment was pooled and compared statistically (1) for differences between the H_2O-treated and drug-treated animals studied during the same time intervals; and (2), using the animals as their own controls, comparing the preliminary 2-hour session in the roller cages with the session subsequent to drug administration. The analysis of variance procedure (Snedecor, 1956) was used to determine if statistically significant differences occurred among the various groups.

Test drugs. The drugs used in this study were the following: *dl*-amphetamine HCl, *l*-amphetamine HCl, *d*-amphetamine SO_4, chlorpromazine HCl, methylphenidate HCl[5], phenmetrazine HCl, phendimetrazine HCl, diethylpropion HCl, methamphetamine HCl, pentylenetetrazol, niketh-amide, caffeine, and the two oxazolines already mentioned. All drugs are indicated as free bases and not as salts.

RESULTS. *Preliminary studies.* Initially, the rats had to be motivated to consume broth regularly and uniformly. This was accomplished by first starving the animals overnight (17 to 24 hours) and then restricting the nutrient source exclusively to broth.

After their initial introduction period the rats were given broth 5 days/week while having available standard rat pellet food and water *ad libitum.*

[3] Generously supplied by Ciba Pharmaceutical Products, Inc.

[4] Generously supplied by Geigy Pharmaceuticals.

[5] Generously supplied by the W. S. Merrell Company.

TABLE 1

Comparison of various anorexigenic drugs in producing an inhibition of broth consumption in rats

Drug	Dose, p.o. (mg/kg)	Measured Response	Quantal Response	
		Mean broth consumption (ml/kg)	No. rats not drinking / No. tested	% Inhibition
McN-742	0	42.7	0/10	0
	2	29.0	1/10	10.0
	4	18.2[a]	2/10	20.0
	8	4.7[a]	7/10	70.0
	10	6.7[a]	7/10	70.0
d-Amphetamine	0	36.1	0/9	0
	2.8	12.0[a]	2/8	25.0
	5.7	13.0[a]	3/7	43.0
	11.4	2.0[a]	7/8	87.5
Amphetamine	0	42.1	0/10	0
	3.2	34.4	0/10	0
	4.7	34.7	1/10	10
	6.3	25.0	2/10	20
	7.9	18.0[a]	2/10	20
l-Amphetamine	0	33.2	0/10	0
	3.9	36.6	0/10	0
	6.3	22.3	0/10	0
	8.7	21.1	0/10	0
	11.0	26.8	0/10	0
	0	33.9	0/10	0
	15.8	21.9	1/10	10
	19.7	27.9	0/10	0
	43.3	7.2[a, b]	3/9	33.3
McN-822	0	23.9	0/11	0
	3	25.9	0/11	0
	6	14.5	4/11	36.3
	9	11.1	5/11	45.4
	12	7.1[a]	7/11	63.6
Methamphetamine	0	36.1	0/9	0
	4	16.4[a]	1/8	12.5
	8	6.6[a]	3/7	43.0
	16	5.1[a]	6/7	85.7
Diethylpropion	0	26.1	0/10	0.0
	14.0	7.7[a]	6/10	60.0
	18.7	10.0[a]	5/10	50.0
	23.3	3.1[a]	8/10	80.0
	28.4	2.6[a]	9/10	90.0
Methylphenidate	0	27.6	0/10	0.0
	8.7	17.1	3/12	25.0
	26.0	7.6[a]	6/11	54.5
	52.0	6.8[a]	7/12	58.3
	104.0	0.0[a, b]	12/12	100.0
Phendimetrazine	0.0	25.6	0/12	0
	21.0	22.2	1/7	14.3
	42.0	14.2	2/7	28.6
	84.0	1.9[a]	6/8	75.0
Phenmetrazine	0.0	30.1	0/8	0
	24.9	19.2	1/8	12.5
	49.8	11.6[a]	2/8	25.0
	99.6	1.6[a]	7/8	87.5

[a] Significantly different from control, $P < .05$.
[b] Toxic dose level.

The rats were then selected for consistency with respect to broth consumption. First, only those rats which immediately began eating when broth was presented were used. Secondly, the animal population plotted against the range in daily broth consumption during a 5-day interval was determined. Arbitrarily, the one-third of the animals showing the greatest range were discarded.

To negate the possibility that the rats were desirous of NaCl and not the nutrient in the broth a 0.9% NaCl solution was given during one period. Under these circumstances the rats began drinking as usual but promptly stopped upon tasting this solution. Vegetable coloring matter was then added to the NaCl solution to approximate the color of the broth since conceivably color could be a factor regulating this feeding. The same results occurred as with the NaCl solution, above.

Comparison of the potencies of various anorexigens. Among the drugs used in this study, it was found that pentylenetetrazol, nikethamide and caffeine did not produce a significant depression of broth consumption until dose levels were reached which obviously impaired normal locomotor activity. Likewise, central depressants also

inhibited this feeding pattern only if motor activity was impaired but not at dose levels below this. Surprisingly, chlorpromazine given orally at a dose level, 5 mg/kg, which produced definite observable ataxia did not decrease broth consumption in rats.

The studies revealed that the anorexigenic drugs depressed desire for broth significantly, and an amplified summary of these findings is shown in table 1. It should be noted that the compounds are arranged in a descending order of potency. As can be seen, a reasonable dose-response pattern was obtained. Furthermore, there was a correlation between the data expressed in a measured (graded) manner and those expressed in a quantal manner.

A summary of a quantal analysis of activity of various anorexigens is given in table 2. The anorexigens are arranged in descending order of apparent potency. In order to determine whether these apparent differences in activity were significant, the dose-response curves were first tested for parallelism. If they were parallel, the differences in apparent activity were then examined to determine if these differences were significant according to the method of Litchfield and Wilcoxon (1949). These data are given under the section headed "significance." In the first column of this section all drugs were compared to the drug with the greatest apparent potency, *d*-amphetamine. No significant difference in potency from this agent is shown by a 0 in line with this drug. If, however, a drug did differ significantly in potency it is denoted by a +. In the second column all subsequent drugs were compared in this manner with the second drug; in the third column a similar comparison was made with the third drug, *etc.*

From these data it is readily apparent that these agents may be divided into three groups with respect to potency in the rat. The most potent drugs are *d*-amphetamine, McN-742, methamphetamine and McN-822. The group of intermediate potency is comprised of amphetamine, diethylpropion, and methyl phenidate, while phenmetrazine and phendimetrazine are the least active of the agents tested in the rat.

It is important to note that duplicate or triplicate ED50 determinations carried out using *d*-amphetamine, methamphetamine and phendimetrazine were highly reproducible.

The findings derived from the use of amphetamine indicate that all or an extremely large percentage of activity is vested in the *d*-isomer. This is indicated by the fact that racemic amphetamine is approximately one-half as active as *d*-amphetamine. This difference in potency is statistically significant. What is of even greater importance is the finding that *l*-amphetamine is totally or in very large part without anorexigenic activity, as indicated in table 1. As shown, a dose of 43.3 mg/kg significantly inhibited broth consumption, but at this dose all animals were extremely hyperactive. Four animals exhibited an

TABLE 2

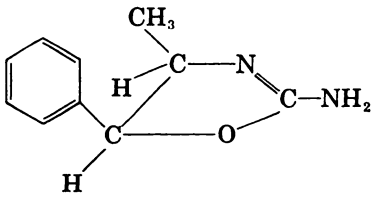
Comparison of oral activity of appetite depressant drugs in rats

Drug	ED50 (mg/kg)	95% Conf. Limits	Significance[a] 1	2	3	4	5	6	7	8
1. *d*-Amphetamine	5.3	3.2–9.0	1							
	6.2	4.0–9.5								
	4.3	2.9–6.3								
2. McN-742	5.8	3.7–9.0	0	2						
3. Methamphetamine	6.0	3.8–9.6	0	0	3					
	5.3	3.7–7.8								
4. McN-822	8.8	5.7–13.7	0	0	0	4				
5. Amphetamine	12.5	7.9–19.7	+	+	+	0	5			
6. Diethylpropion	15.5	12.2–19.7	+	+	+	+	0	6		
7. Methyl phenidate	26.0	11.3–59.5	+	+		+	0		7	
8. Phenmetrazine	53.0	29.4–95.3	+	+	+	+	+	+		8
9. Phendimetrazine	59.0	33.4–104	+	+	+	+	+	+	+	0
	59.5	36.1–98.2								
	59.5	42.7–83.0								

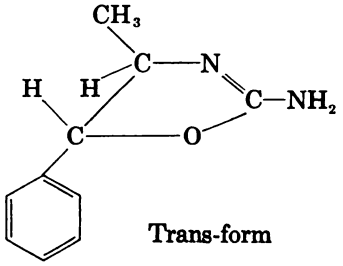
[a] Significance with respect to ED50 values—see RESULTS.

intermittent tremor and one died. In spite of this, 6 of the animals still persisted in drinking the broth.

While, with the above optical isomers, activity appears to be isomer-dependent, no statistically significant difference in the anorectic activity of a pair of geometric or cis, trans-, isomers was noted. The original compound, McN-822, was the cis-form and subsequently, the trans-form was synthesized. These are shown below.



Cis-form
(McN-822)



Trans-form

The ED50 of the cis-form was 8.8 (5.7 to 13.7) mg/kg while the trans-form ED50 was 7.0 (4.7 to 10.5) mg/kg.

Methyl phenidate appears to be somewhat different from other anorexigens in that its dose-response curve is flatter than those seen with other anorexigens. Although no significant deviation from parallelism of the dose-response curves was observed between some of the anorexigens and this agent it was observed with others. Hence, no comparison of potency could be made between methyl phenidate and methamphetamine, diethylpropion, or phenmetrazine.

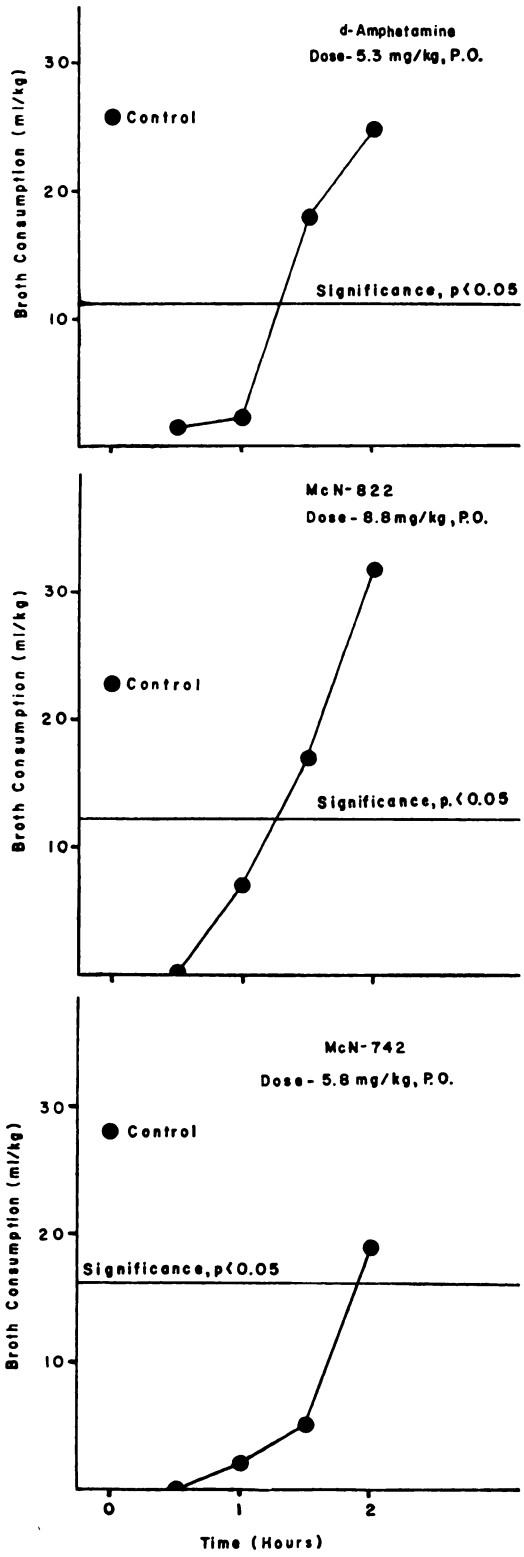
In one experiment *d*-amphetamine was administered subcutaneously to ascertain that depression of broth consumption was due to a systemic drug action and not to a physical effect on the gastrointestinal wall of the rat, which in turn would produce an anorectic effect. A dose of 1.5 mg/kg, subcutaneously, significantly depressed feeding when compared to orally or subcutaneously treated controls.

Determination of duration of anorexigenic action. Studies were carried out with McN-742 and McN-822 to determine if these agents differed from *d*-amphetamine with regard to the duration of the anorexigenic activity. These substances were administered at predetermined ED50 dose levels to 4 groups at 30, 60, 90 and 120 minutes prior to broth administration along with a control group receiving a comparable volume of water 30 minutes prior to testing.

The results of these findings are summarized graphically in figure 1, where broth consumption in ml/kg is plotted against time. McN-822 and *d*-amphetamine produced a statistically significant decrease in broth consumption at the 30- and 60-minute intervals. McN-742 was longer acting in that it produced a significant decrease in broth consumption after 30, 60 and also 90 minutes.

Assessment of drug-induced changes in locomotion and behavior. Close observation of rats receiving oral doses of anorexigens ranging below and somewhat above the ED50 did not reveal the presence of untoward, incapacitating responses. With the higher doses there appeared greater locomotion and a constant searching action. This activity was particularly evident with methyl phenidate; and, although this agent does produce true anorexia, it would appear that the margin between incapacitating dose levels and anorexigenic dose levels is narrower than with other anorexigens.

To substantiate further the above gross subjective observations that, with the other drugs, excessive incapacitating motor stimulation did not occur at anorexigenic dose levels, rats were placed in "roller cages" and increase in rotation of these cages was recorded. In the two water-treated control groups a slight decrease in mean rotation was seen when the second 2-hour period was compared to the initial 2-hour period. A decrease in rotations from 47 to 29 was observed in the first study and a decrease from 48 to 20 rotations was observed in the second. In the animals treated with anorectic ED50 amounts of drug rotation were always increased. However, only methamphetamine and phenmetrazine significantly increased rotation when a comparison was made between these drug-treated groups and the corresponding water-control group. When compared to the respective second hour control activity (29 and 20), the methamphetamine treated animals produced a mean rotation of 200, while



the phenmetrazine treated group displayed a mean rotation of 123.

It should be pointed out that, although an increase in activity was apparent, this increase was very moderate and well within the physical capacity of the animals. In most instances, less than one rotation/minute was made under the influence of the drug.

DISCUSSION. In the present study rats were confronted with making a choice between two kinds of available foods, *viz.*, rat pellet food and beef broth. That these animals immediately and invariably chose beef broth and ignored available pellet food indicates a feeding preference on their part which is strongly suggestive of appetitive behavior in humans. Likewise, it is suggested that drug suppression of feeding in these experiments approximates a suppression of "appetite" as distinct from "hunger." This should not be construed to indicate a selective suppression of "appetite" rather than of "hunger"; in all probability the drugs studied indiscriminantly suppress all feeding behavior.

In absolute terms, proportionally larger amounts of drug were employed experimentally than would be used clinically. Nonetheless, it would appear that, from a comparison of anorexigenic potencies of various drugs tested, there is an agreement in the relative order of potency seen in this test and that which is observed clinically.

The finding that *d*-amphetamine was the active anorexigenic component of racemic amphetamine agrees with the clinical observation first noted by Rosenberg (1942) and later by Colton (1943), Williams *et al.* (1948), and Ferguson (1949). Williams *et al.* (1948) also tested *l*-amphetamine in patients and came to the conclusion that this material was much less active as an anorexigen than *d*-amphetamine or amphetamine.

Many other experimental studies may be cited which indicate that biological activity preferentially occurs with one of a pair of optical isomers. Likewise, instances have been cited which indicate that preferential biological activity may occur with one of a pair of geometric isomers (Adam-

FIG. 1. Effect of oral administration of ED50 amounts of *d*-amphetamine, McN-822 and McN-742 on duration of significant inhibition of appetite as measured by depression of broth consumption.

The points which are mean values and are below the line designated "significance" are significant at P value of .05 or less while points above line are not.

son *et al.*, 1951; Beckett, 1960; Schueler, 1960). It would appear that no such specificity is apparent with geometric isomers in the case of McN-822.

Several new chemical agents have been introduced recently into clinical practice as anorexigens and these have been reviewed from a clinical and pharmacological standpoint by Friedman (1960). Among them are phenmetrazine (Berneike, 1954), diethylpropion (Brunchow, 1959), and phendimetrazine (Stegen *et al.*, 1960). Although these agents are effective, no distinct advantages over *d*-amphetamine are apparent; they are all less active than *d*-amphetamine. In this connection, McN-742 may have advantages over *d*-amphetamine as an appetite suppressant since, in addition to being as potent as *d*-amphetamine, it also displayed a longer duration of action.

Although appetite suppression observed with methyl phenidate is in agreement with the findings of Karczmar and Howard (1959), as shown in the present study, this substance appears to display greater central stimulant effects in relation to its anorexigenic activity than do other anorexigens.

The findings seen with methyl phenidate and other central nervous system stimulants indicate that, although no anorexigenic substance has been seen which is devoid of central stimulant properties, central nervous stimulants *per se* do not induce anorexia. Likewise the degree of central stimulation of the anorexigens at effective dose levels appears to be similar, there being at most only very small differences among the substances tested.

Until now, the best known and seemingly most effective anorexigens have been agents containing a phenethylamine-like chemical configuration or agents, like phenmetrazine or methyl phenidate, in which the ethylamine side chain was closed to form a ring. This study demonstrated that at least two oxazoline compounds show striking and selective anorexigenic activity.

SUMMARY

A simple quantitative test procedure for assessing the anorexigenic potential of drugs is described. The data presented suggest that this test procedure has as an end point preferential feeding behavior and may, as a consequence, be a test which measures drug-induced suppression of "appetite."

Of 10 drugs tested 4 were very potent: *d*-amphetamine, methamphetamine, and two oxazolines, McN-742 (2-amino-5-phenyl oxazoline) and McN-822 (2-amino-4-methyl-5-phenyl oxazoline). A group of drugs of intermediate potency consisted of *dl*-amphetamine, diethylpropion, and methyl phenidate. Phenmetrazine and phendimetrazine displayed slight potency, and *l*-amphetamine was inactive.

Of the drugs tested, the oxazolines appear to be very interesting in that McN-742, in addition to being as potent an anorexigen as *d*-amphetamine, is longer acting than this substance.

In addition to the quantification of anorexigenic potency, the test procedure demonstrated that, at effective dose levels, all of the anorexigens display a similar degree of central nervous system stimulation. It appears that a drug, to be vested with anorexigenic activity, must also be a central nervous stimulant. However, not all central nervous stimulants are anorexigens.

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