

Modified Method for Studying Anorexigenic Agents in Dogs

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Mongrel dogs, selected to accept a food offering every 10 minutes for 8 hours and to respond reproducibly to oral test doses of dextroamphetamine sulfate, have been employed in a test procedure designed to evaluate anorexigenic agents quantitatively. Linear dose-response and dose-duration curves have been obtained, demonstrating the validity of the procedure and affording the possibility of quantitative comparisons of potency and duration of anorectic activity of various drugs.

SEVERAL METHODS of testing anorexigenic agents in dogs have been described in the literature. Various end points, such as reduced consumption of food (1), complete refusal of food (2), and weight loss (3) have been employed to ascertain anorectic activity of test compounds. No attempts were made to obtain dose-response curves or time-action curves in order to compare potency and duration of action of different compounds. These methods are therefore qualitative or at best semiquantitative. Wendel *et al.* (4) described a quantitative test procedure for determining time-action curves of anorexigenic agents in sustained-release forms. A modification of their method is reported in this paper.

The objectives of this study were twofold: first, to establish a test in which reproducible responses could be obtained in unoperated animals under normal physiological conditions; and second, to establish a test that would be pharmacologically valid as shown by (a) a linear relationship between the log of the dose of a drug and the biological response elicited, (b) a linear relationship between the log of the dose and the duration of action of the dose, and (c) observing the desired biological activity at dosage levels far below those eliciting toxic side effects.

METHODS

Animal Selection.—Healthy mongrel dogs of either sex, weighing between 10 and 20 Kg., were tested and selected by the following criteria.

Each animal, after 18 hours without food, accepted $\frac{1}{24}$ lb. of canned dog food every 10 minutes for 8 hours, then consumed an additional 1 lb. of food (feeding control). Each animal showed some degree of anorexia at a dose of 0.75 mg./Kg. of dextroamphetamine sulfate. No animal was so sensitive that it refused all six food offerings during any hour beyond the fifth hour at a dose of 0.4 mg./Kg. of dextroamphetamine sulfate.

Test Procedure.—A working colony of 10 animals selected from 17 dogs was maintained throughout the study. Frequent feeding controls and drug controls were conducted on the colony to insure reproducibility of the responses. During a feeding control each animal was offered $\frac{1}{24}$ lb. of dog food every 10 minutes for 8 hours. An animal that did not accept every food offering was replaced. For the drug controls, the animals received orally one of the selected doses of dextroamphetamine sulfate (0.4, 0.6, or 0.75 mg./Kg.) and were then offered food every 10 minutes for the subsequent 8 hours. The anorectic response of the whole colony and the responses of the individual animals were checked;

any animal failing to conform fully was replaced. In addition, animals that showed marked and persistent changes in their patterns of refusal after receiving one of the standard doses of dextroamphetamine sulfate were replaced. Only by taking such precautions can valid and reproducible results be obtained.

The drug, in capsules, was included in the first food offering. If any food offering was not consumed, it was removed from the cage and reoffered. In this way no animal was presented with more than one food offering at a time. To facilitate the evaluation of the data, the following calculation was employed:

$$\% \text{ Anorexia for each hr. interval} = \frac{\text{Total No. of refusals of colony during this hr.} \times 100}{\text{Total No. of offerings to colony}}$$

The colony was used not more than 1 day a week.

TABLE I.—TYPICAL ANORECTIC RESPONSES OF THREE ANIMALS TO 0.6 mg./Kg. OF DEXTROAMPHETAMINE SULFATE*

Animal No.	Date	Intervals Post Drug, Hr.							
		1	2	3	4	5	6	7	8
2	11/15/61	0	5	6	6	6	6	6	5
	11/29/61	2	6	6	6	6	6	6	4
	1/3/62	1	6	6	6	6	6	5	0
7	11/15/61	0	3	6	6	6	0	0	0
	11/29/61	0	4	6	6	1	0	0	0
	1/3/62	0	5	6	6	3	0	0	0
29	11/15/61	0	3	6	6	6	6	6	5
	11/29/61	2	6	6	6	6	6	6	5
	1/3/62	0	5	6	6	6	6	6	5

* Numbers in the table show the number of refusals in any hourly interval.

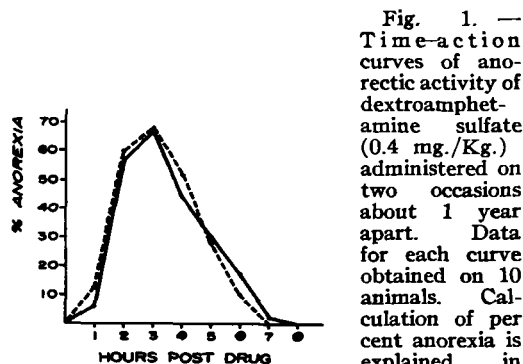


Fig. 1. — Time-action curves of anorectic activity of dextroamphetamine sulfate (0.4 mg./Kg.) administered on two occasions about 1 year apart. Data for each curve obtained on 10 animals. Calculation of per cent anorexia is explained in text. Key: — May 31, 1961; - - - May 16, 1962.

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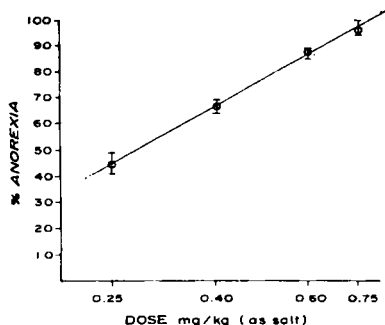


Fig. 2.—Dose-response curve for graded doses of dextroamphetamine sulfate administered orally to dogs. Peak activity in all cases occurred at 3 hours post drug. Confidence limits (95%) of the line are shown.

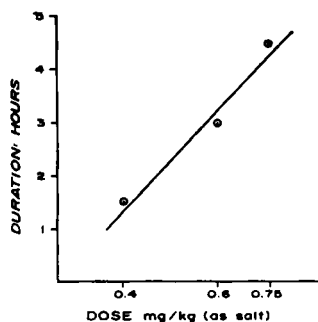


Fig. 3.—Dose-duration curve for graded doses of dextroamphetamine sulfate administered orally to dogs. The duration in hours required to descend from the peak to 50% anorexia has been plotted against the log of the dose.

TABLE II.—SUMMARY OF DATA OBTAINED IN THIS STUDY

Drug	Dose, mg./Kg. as Salt	Dogs per Trial, No.	Trials, No.	Max. Responses, % Anorexia	Duration of Action, Hr. ^a	Relative Areas under Time-Action Curves
Dextroamphetamine sulfate	0.25	10	2	45	...	1.0
	0.40	10	3	67	1.5	2.1
	0.60	10	3	88	3.0	3.3
	0.75	10	2	96	4.5	>4.0
<i>l</i> -Amphetamine sulfate	2.50	10	2	86	2.8	3.0

^a For the purposes of this study duration of action was defined as the time required for anorectic activity to descend from the maximum to 50% anorexia.

RESULTS AND DISCUSSION

Since the aims of this study were to develop a test procedure that would give reproducible results and be pharmacologically valid, the results will be discussed accordingly.

Reproducibility.—*Individual Animals.*—There was considerable individual variation in the response to the anorectic agents. This, however, appeared to be of minor importance as long as responses of the single animal and the colony as a whole were consistent. Nevertheless, the pattern of refusals was usually remarkably constant as shown in Table I for three typical dogs on 3 different days.

Colony as a Whole.—The reproducibility of the colony as a whole tested on two occasions about 1 year apart is summarized in Fig. 1.

Validity.—The linearity of the log dose-response relationship obtained with four doses of dextroamphetamine sulfate clearly demonstrates that the colony functioned as a pharmacologically valid test object (Fig. 2). Since the processes (redistribution, metabolism, excretion) responsible for decreasing drug concentration at the site of action are usually first-order processes, the decrease in the effective concentration of a drug is exponential. Therefore, the time required for the peak response to descend to some selected level should be linearly related to the log of the dose. Thus, the relationship between log dose and duration of action has been examined with our test procedure and was found to be linear (Fig. 3). Some of the pertinent data are summarized in Table II. A plot of relative areas under the time-action curves (total effectiveness) against log dose will also yield a linear relationship, which constitutes additional proof for the validity of the test

procedure. Recently, Friedman (5) stated that the potency of the anorexigenic effect of *l*-amphetamine sulfate had not been evaluated. In our test procedure it required almost exactly four times as much of the *levo* form (2.5 mg./Kg.) to produce the same maximum anorexigenic activity as 0.6 mg./Kg. of the *dextro* form, and the total effectiveness (area under the time-action curve) was also nearly equal.

Tolerance to the anorexigenic activity of dextroamphetamine sulfate was not seen; undesirable side effects, such as excitation and salivation, occurred only rarely at the highest dose used (0.75 mg./Kg.).

SUMMARY

A test procedure in dogs for the quantitative evaluation of anorexigenic agents has been described. Strict adherence to the criteria established yields reproducible responses and linear log dose-response relationships, indicating that the procedure and the test object are pharmacologically valid. Anorectic activity can be demonstrated at doses well below those producing marked signs of CNS stimulation. Time-action curves of different agents and of several doses of the same agent can be obtained. Therefore, the test procedure appears to be extremely useful for comparing the relative potencies and durations of action of anorexigenic agents.

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