Behavioral tolerance of squirrel monkeys to hypoxia: A model for evaluating drug therapy*

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A behavioral tolerance-time procedure for measuring hypoxia-induced disruption of avoidance behavior was developed using squirrel monkeys (*Saimiri sciureus*) under a Sidman avoidance contingency. Hypoxic atmospheres were acutely and repeatedly presented to highly trained monkeys during avoidance sessions with 5 min of normoxia between presentations of hypoxia. Behavioral tolerance time was defined as the period from the initiation of hypoxia to the occurrence of the first shock during each hypoxic presentation. Parametric studies demonstrated that reliable behavioral tolerance times could be obtained using a 5-sec response-shock interval and a 7% O₂ atmosphere. Acetazolamide, a drug previously shown to be beneficial for several types of functioning under hypoxic conditions, markedly lengthened behavioral tolerance time, suggesting a valid model for evaluating drug therapies.

"Tolerance time" is often used as a dependent variable in the study of the effects of hypoxic environments on living organisms. The definition of tolerance time covers the interval from initiation of the hypoxic condition to an endpoint that is identical for all Ss. Physiological and pharmacological investigations have often used death as the endpoint, although other easily measured endpoints are loss of consciousness and drops in body temperature. Death has the obvious disadvantage of preventing repeated measurements using the same S. Loss of consciousness and large drops in body temperature may have gross nonreversible or long-term effects (Fregley, 1954). A preferable method of measuring tolerance to hypoxia would be one which left the animal relatively intact, so that reliable repeated measurements could be taken.

An alternative measure involves a behavioral definition. Such a definition should involve an endpoint which is meaningful in terms of behavior and in terms of the environmental variable being tolerated. A behavioral schedule which fulfills these requirements is Sidman (nondiscriminated) avoidance (Sidman, 1953). This schedule involves the organism's repeatedly postponing the occurrence of an aversive stimulus,

e.g., electric shock, by pressing a lever. The schedule produces a relatively constant response rate which may be maintained over long periods of time (Mason, Brady, & Tolliver, 1968). In well-trained primate Ss, the occurrence of a shock is rare. Therefore, the endpoint of the tolerance time may be defined as the occurrence of an electric shock. Such an endpoint is meaningful in terms of the behavioral schedule because it represents the failure of the monkey to respond at a rate which would maintain avoidance. The endpoint can also be related to other investigations of the effects of hypoxia on operant behavior since one of the most frequent effects of hypoxia is a reduction in response rate (Annau & Weinstein, 1967; Buckley, Solaro, & Barry, 1969). In addition, the Sidman avoidance schedule allows continuous evaluation of changes in response rate both before and after the occurrence of an electric shock. By changing from hypoxia to normoxia upon delivery of the first electric shock, recovery of response rate from the decremental effects of hypoxia may be measured. Since the decremental effects of acute hypoxia on response rate usually recover rapidly, repeated measures of tolerance time can be taken within the same experimental session.

This paper will discuss: (1) the development of a behavioral tolerance time procedure for measuring hypoxia-induced disruption of Sidman avoidance behavior in squirrel monkeys (*Saimiri sciureus*), its procedural variables and limitations; and (2) methodological considerations concerning the use of this procedure for the evaluation of potential drug therapies.

EXPERIMENT 1: RELIABLE MEASUREMENT OF BEHAVIORAL TOLERANCE TIME

The development of a predictive model necessarily involves the development of a reliable measuring system. During the initial exploratory studies, it became obvious that the schedule parameters originally chosen to train the Ss [20-sec response-shock (R-S) interval, 5-sec shock-shock (S-S) interval] yielded an intolerable degree of variability in our measurements. Therefore, a parametric experiment was performed in order to determine values for the R-S interval which would yield reliable tolerance-time measurements.

Method

Subjects. Two adult male squirrel monkeys were used. Both monkeys had previously been trained on a FR 30, FI 10 multiple shock-avoidance schedule and had been intermittently exposed to varying degrees of hypoxia over the year prior to the present experiments.

Apparatus. Monkeys were trained in a squirrel monkey chair similar to that described by Herd, Morse, Kelleher, and Jones (1969). In this chair, the monkey was restrained by waist and tail stocks with a Lehigh Valley Model 1352 rat lever located at chest height. An electric shock (60 Hz, 6 mA, 0.1 sec) could be delivered through the tail stock. A rectangular Plexiglas gas chamber enclosed the monkey's body above the waist. The experimental atmosphere was regulated by a solenoid valve system and O₂-N₂ cylinders. The volume of the gas chamber minus the volume of the monkey's body was approximately 1 liter. The time to change the chamber atmosphere completely was calibrated to be approximately 30 sec at a flow rate of 15 liters/min. Experiments were run in a sound-attenuated chamber; standard solid-state programming and control equipment was used.

Design. Behavioral tolerance to 8% O₂ was tested under three values of the R-S interval of the Sidman avoidance schedule (20, 10, 5 sec). Ten tolerance times were measured per testing session. Each value of the R-S interval was tested during five consecutive daily sessions.

Training. Both monkeys were initially trained on a Sidman avoidance schedule with a 20-sec R-S interval. Throughout all experiments, the S-S interval was 5 sec. Each training session lasted 1 h. Training was continued until one or zero shocks occurred in a given session. At this point, tolerance-time testing sessions were initiated for the 20-sec R-S interval value (see below). After

*Experiments reported herein conformed with the standards of the "Guide for Laboratory Animal Facilities and Care."

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Fig. 1. Mean and standard error of 10 behavioral tolerance-time trials measured in two monkeys during five consecutive daily sessions under 20-, 10-, and 5-sec R-S intervals. Note the decreased variability with decreased R-S interval.

completion of 5 days of such testing, training was initiated under the 10-sec R-S interval and was continued until one or zero shocks occurred. After 5 days of tolerance-time testing under the 10-sec R-S interval, training was initiated under the 5-sec R-S interval to the same criterion.

Tolerance-time testing. Each tolerance-time testing session began with a 30-min "warm-up" period during which responding occurred in a normoxic (21% O_2) atmosphere. Each tolerance-time trial consisted of presenting the experimental (8% O_2) atmosphere and measuring the time between the initiation of hypoxia and the presentation of the first shock. The occurrence of a shock resulted in the rapid representation of the normoxic atmosphere and an intertrial interval of 5 min, during which responding continued. This cycle was repeated until 10 tolerance-time trials were completed.

Results

Figure 1 shows the mean and standard error of the mean tolerance time for each testing session under each R-S interval. Each monkey's data are plotted separately. Inspection of this figure indicates that both mean and standard error of the tolerance time decreased with decreasing R-S interval. An arbitrary cutoff point of 1,800 sec had to be used under some conditions, since the monkey's response rate did not show enough of the decreasing trend to warrant further running. In addition, extremely long tolerance times would not be practical if repeated measurements were desired. As a result of this data, the

5-sec R-S interval was chosen for subsequent experiments since it yielded the least intra- and intersession variability.

EXPERIMENT 2 EFFECTS OF VARYING DEGREES OF HYPOXIA ON BEHAVIORAL TOLERANCE TIME

The purpose of this experiment was to evaluate the relationship between the percent O_2 and behavioral tolerance time in order to determine a suitable degree of hypoxia to be used in future studies.

Method

Subjects. Five male squirrel monkeys were used. Two had previously served as Ss for Experiment 1. Three new monkeys had been trained to criterion on a Sidman avoidance schedule using a 5-sec R-S interval.

Design and procedure. Four levels of hypoxia were used: 13%, 11%, 9%, and 7% O_2 . Each level of hypoxia was presented on three consecutive tolerance-time trials during a single experimental session for a total of 12 trials. Each trial was separated by a 5-min intertrial interval. The initial "warm-up" time was 10 min. The cutoff point for each trial was reduced to 600 sec.

Results

Mean tolerance time for each monkey, and for the group, is shown in Fig. 2 as a function of the percent O_2 . Mean tolerance time decreased with increasing degrees of hypoxia. However, with 13%, 11%, and even 9% O_2 , some Ss occasionally tolerated hypoxia until the cutoff point was reached. It was only at 7% O_2 that reliable low tolerance times were obtained. Therefore, subsequent investigations involving attempts to extend behavioral tolerance time using drugs used only the 7% O_2 atmosphere.

EXPERIMENT 3: SENSITIVITY OF BEHAVIORAL TOLERANCE TIME TO DRUG THERAPY

Acetazolamide

Experiments 1 and 2 developed a reliable system for measuring hypoxia-induced disruption of avoidance behavior. But before this procedure could be used to evaluate potential drug therapies, validation studies were required. Such validation involved a comparison of the effects of drugs on behavioral tolerance time with their effects on external criteria. In the present investigation, such validation involved evaluating the effects of acetazolamide, a carbonic anhydrase inhibitor previously

Fig. 2. Mean of three tolerance-time trials under four degrees of hypoxia. Each point represents mean behavioral tolerance time for one monkey under one degree of hypoxia. The solid curve represents the group means.

demonstrated to reduce hypoxia-induced visual decrements (Kobrick, 1970), attenuate respiratory alkalosis and hypocapnia associated with hypoxia-induced hyperventilation and alleviate symptoms of acute mountain sickness (Cain & Kronenberg, 1969; Landowne, Forward, & Hansen, 1969). It was assumed that significant increases in behavioral tolerance time under hypoxic conditions which resulted from administration of acetazolamide would provide evidence for the predictive validity of the behavioral tolerance-time model, at least for the evaluation of chemotherapeutics.

Method

Using the same five monkeys and a similar testing procedure as in Experiment 2, five behavioral tolerance-time trials were presented to each monkey on 4 consecutive days. On Days 1, 3, and 4, a 0.4-cc normal saline placebo was injected IP 2 h before the testing session. Two 25-mg/kg injections of acetazolamide were administered IP 20 and 2 h before testing on Day 2.

Results and Discussion

Means of the five tolerance-time trials for each monkey and the group mean for each session are shown in Fig. 3. The therapeutic effect of acetazolamide is evident. Tolerance time markedly increased for all monkeys during Session 2 and returned to near predrug levels by Session 4. Since several monkeys reached the 600-sec cutoff point on one or more trials during Session 2, the data presented in Fig. 3 probably represent an attenuation of the extent of the drug's therapeutic effect. That is, some Ss would have tolerated hypoxia longer if allowed to do so during Session 2.

Fig. 3. Mean of five tolerance-time trials during four consecutive daily sessions. A saline placebo was injected 2 h prior to Sessions 1, 3, and 4. Injections of acetazolamide (25 mg/kg, IP) were administered 20 and 2 h prior to Session 2. Each point represents the mean behavioral tolerance time of one monkey during one session. The solid curve represents the group mean. Note the marked increase in behavioral tolerance time following drug administration and subsequent return to near predrug levels.

The behavioral tolerance-time model, using the squirrel monkey as the S, appears to be a reliable and convenient method for measuring hypoxia-induced disruption of Sidman avoidance behavior. The method allows repeated measurements on the same S, while minimizing the chances of acclimatization by presenting only highly acute exposure to hypoxia. This technique also has the advantage of minimizing the number of shocks

presented to the S, since the first shock presented under hypoxic conditions on each trial leads to the rapid presentation of a normoxic atmosphere. The resulting rapid improvement in performance minimizes the probability of residual toxic or disruptive effects of prolonged periods of shock and/or hypoxia.

The apparent large increase in behavioral tolerance under the drug condition illustrates one possible disadvantage of the procedure as presently reported. That is, a drug may increase the behavioral tolerance to 7% O₂ to such an extent that the monkey repeatedly reaches the cutoff point. This situation does not allow the measurement of differences in high degrees of tolerance. If such measurement is critical, the procedure must then be modified to either extend the period before cutoff on each trial, or to increase the degree of hypoxia in the experimental atmosphere. Various studies in our laboratory indicate that if a particular degree of hypoxia is tolerated for 600 sec, the monkey will generally continue to perform "indefinitely." Therefore, extending the maximum length of each trial would not be advantageous. Reducing the oxygen concentration in the experimental atmosphere would probably be a more useful modification.

The present model does not simulate acute mountain sickness or other disorders resulting from prolonged exposure to hypoxia. For example, the onset of symptoms of acute mountain sickness generally occurs after several hours of exposure. The use of highly acute hypoxic exposures raises questions concerning the face validity of attempting to predict from the laboratory model to acute mountain sickness and related disorders. However, the results of Experiment 3 suggest that the behavioral tolerance-time model may be empirically useful in evaluating

therapeutic agents which might then be used in chronic experiments. The combination of acute and chronic experiments, involving both behavioral and physiological measurements, would be necessary for a full evaluation of any therapeutic regime.

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