

DEVELOPMENT OF TOLERANCE IN RATS TO THE HYPOTHERMIC EFFECTS OF  
D-AMPHETAMINE AND APOMORPHINE

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SUMMARY

Rats given d-amphetamine (15 mg/kg i.p.) or apomorphine (10 mg/kg i.p.) and placed in a cold environment (4°C) developed marked hypothermia. After daily injections of either drug for seven weeks, the maximal hypothermic responses to d-amphetamine or apomorphine were reduced to 72% and 19% of those obtained initially. Subsequent injection of ET-495, a central dopamine receptor stimulant, caused rectal temperature to decrease only 72% and 49% as much as in control animals. The hypothermic response to apomorphine was also depressed in d-amphetamine-treated animals. These observations suggest that the tolerance to the hypothermic effects of both d-amphetamine and apomorphine that develops is due at least in part to alterations in the sensitivity of dopamine receptors.

Administration of d-amphetamine to rats placed in a cold environment causes a profound hypothermia (1). This effect is also produced by apomorphine (2), but is absent in animals pretreated with drugs that block the central dopamine receptors (2), intraventricular or intracisternal 6-hydroxydopamine (2), or lesions that destroy the mesolimbic dopaminergic projections to the olfactory tubercles (2; Yehuda and Wurtman, submitted for publication). Hence the hypothermic effect presumably results from the release of dopamine and the consequent activation of dopamine receptors in limbic structures.

There is some controversy in the literature concerning the

extent to which laboratory rodents develop tolerance to the behavioral and physiological effects of d-amphetamine (3). Lu et al. (4) observed that rats given 16 mg/kg of the drug developed tolerance to its anorexic effects; Lewander (5) found that rats kept at room temperature and given 16 mg/kg of d-amphetamine for 12-14 days, and then 32 mg/kg for 12-14 days, showed tolerance to both the anorexia and the hyperthermia that it produced. However, Lewander (5) and Ellinwood et al. (6) did not observe tolerance to the increased motor activity or stereotyped behavior produced by comparable doses of d-amphetamine.

The following observations indicate that partial tolerance to the hypothermic effect of d-amphetamine does develop. This tolerance does not appear as rapidly as the tolerance to comparable doses of apomorphine, but appears to be mediated by a similar mechanism: the altered sensitivity of post-synaptic dopaminergic receptors.

#### Materials and Methods

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Mass.) initially weighing  $115 \pm 6$  g were placed in individual wire-mesh cages (20 × 24 × 17 cm) in a well-ventilated room at an ambient temperature of 22°C and relative humidity of 45% for the duration of the experiment. Food (Big Red Lab Chow, Agway, Inc., Syracuse, N.Y.) and water were available at all times. Light ("Vita-Lite," Duro-Test Co., North Bergen, N.J.) was provided between 9:00 a.m. and 9:00 p.m. daily. Animals were weighed and were then given daily intraperitoneal (i.p.) injections of 1 ml of saline (four rats), solutions of d-amphetamine sulfate (15 mg/kg free base; Smith, Kline & French Co.; six rats), or apomorphine hydrochloride (10 mg/kg free base; Smith, Kline & French Co.; six rats) between the hours of 10:00 a.m. and 5:00 p.m.

On the first day of the experiment, animals were transferred to individual plastic cages and their colonic temperatures measured with a telethermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio) and recorded; immediately thereafter they received their initial injections. They were then placed in the climate chamber preset to 4°C and to a relative humidity of 45%. Colonic temperatures were measured at half-hour intervals for 90 min. This procedure was repeated weekly for seven weeks.

At the end of the seven-week experimental period, all animals received ET-495 (7-[2"-pyrimidyl]-4-piperonyl-piperazine; 100 mg/kg i.p.; Servier), a dopamine receptor stimulant (7), suspended in methyl cellulose. Two previously-untreated rats were injected with methyl cellulose to provide controls for this diluent. On the following day, all of the animals that had received ET-495 were injected with apomorphine hydrochloride (10 mg/kg free base i.p.).

Two additional experiments were performed to confirm the development of tolerance to d-amphetamine hypothermia. In the first, eight rats received d-amphetamine sulfate (15 mg/kg free base i.p.) and six received saline daily for four weeks. In the second, seven rats received d-amphetamine sulfate (15 mg/kg free base i.p.) and five received saline daily for three weeks.

The hypothermic responses of the animals in each group to daily injections given for different periods were compared using a paired Student's t-test; the responses of different groups to different treatments were compared with a two-sample Student's t-test. The d-amphetamine was a gift of Dr. Harry Green, Smith, Kline & French Co.; the apomorphine was generously provided by Dr. George Cotzias, Brookhaven National Laboratories.

### Results and Discussion

Rats receiving saline alone exhibited some hypothermia ( $-0.85 \pm 0.15^{\circ}\text{C}$ ) after the first week of injections; this response became insignificant thereafter. Initially, the doses of d-amphetamine or apomorphine produced comparable levels of hypothermia ( $-4.02$  vs.  $-4.00^{\circ}\text{C}$  respectively; Fig. 1); rectal temperatures in both groups differed significantly from those of saline-treated rats. Maximum hypothermia was observed 60 min after administration of d-amphetamine and 30 min after apomorphine (Fig. 1).

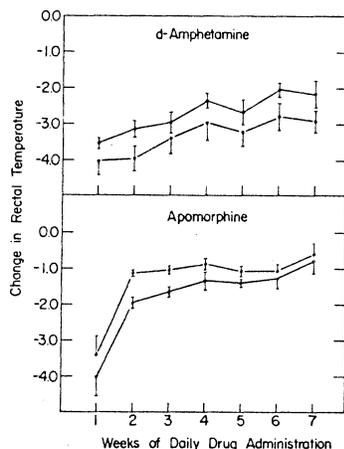


FIG. 1

Fall in rectal temperature 30 (o) or 60 (●) min after rats received d-amphetamine sulfate (15 mg/kg free base i.p.) or apomorphine hydrochloride (10 mg/kg free base i.p.), and were placed in a chamber at  $4^{\circ}\text{C}$ . Groups of animals received each drug daily for up to seven weeks. At the end of each week, rectal temperature was measured. Data are given as mean  $\pm$  standard error of the mean. Control animals given daily injections of saline and placed in the cold exhibited a slight fall in rectal temperature during the first week of injections ( $-0.85 \pm 0.15^{\circ}\text{C}$ ), but no change thereafter.

After three weeks of daily injections, d-amphetamine became 84% as effective in producing hypothermia as during the first week, while apomorphine was 41% as effective. After seven weeks of daily injections, d-amphetamine was 72% as hypothermic as initially ( $-2.90$  vs.  $-4.02^{\circ}\text{C}$ ), while apomorphine was only 19% as ef-

fective ( $-0.78$  vs.  $-4.00^{\circ}\text{C}$ ) (Fig. 1.).

In the second experiment, the rectal temperatures of animals that had received d-amphetamine for four weeks fell by only 32% as much as after the first week of injections ( $-1.43$  vs.  $-4.20^{\circ}\text{C}$ ). In the third experiment, animals treated daily for three weeks exhibited only 61% of the response to d-amphetamine that they had shown initially ( $-1.97$  vs.  $-3.22^{\circ}\text{C}$ ). All groups thus demonstrated tolerance to d-amphetamine, although this phenomenon developed at different rates in the three experiments.

At the end of the seven-week experimental period, animals that had previously received daily injections of saline exhibited a marked fall in rectal temperature after treatment with ET-495 ( $-5.03 \pm 1.07^{\circ}\text{C}$ ); this fall became maximal after 90 min. The animals that had previously received d-amphetamine showed a temperature response that was only 72% as great ( $-3.60 \pm 0.86^{\circ}\text{C}$ ), while those that had been treated daily with apomorphine showed a fall in rectal temperature that was only 49% as great ( $-2.50 \pm 0.88^{\circ}\text{C}$ ). (The control rats injected with methyl cellulose did not exhibit significant hypothermia.) On the following day, rats that had been treated chronically with d-amphetamine were injected with apomorphine and exhibited significantly less hypothermia ( $-1.90$  vs.  $-2.45^{\circ}\text{C}$ ) than control, saline-injected animals.

The observations that partial tolerance develops after seven weeks to the hypothermic effects of both d-amphetamine and apomorphine (Fig. 1); that animals partially tolerant to d-amphetamine show decreased responsiveness to apomorphine or ET-495; and that d-amphetamine hypothermia results from the release of dopamine (2) and the activation of receptors receiving synapses from mesolimbic neurons (2; Yehuda and Wurtman, submitted for publication), are all compatible with the hypothesis that this tolerance at

least partially reflects alterations in the sensitivity of central dopamine receptors, perhaps in the nucleus accumbens or olfactory tubercle. The difference in the rate at which tolerance develops to the hypothermic effects of d-amphetamine and apomorphine could reflect differences in the rates at which the drugs are metabolized after chronic administration, or perhaps progressive decreases in the amounts of pre-synaptic dopamine released by d-amphetamine. Further experiments will be needed to evaluate the contributions of such factors to d-amphetamine tolerance. Lesions destroying the terminals of mesolimbic dopaminergic neurons block both the hypothermia and the stereotypy caused by d-amphetamine administration (Yehuda and Wurtman, submitted for publication). Thus it is perhaps surprising that tolerance develops to the former effect (Fig. 1) but not, reportedly (5, 6), to the latter.

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