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Differential Effects of Amphetamine and Related Compounds on Locomotor Activity and Metabolic Rate in Mice

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BUSHNELL, P. J. *Differential effects of amphetamine and related compounds on locomotor activity and metabolic rate in mice.* PHARMACOL BIOCHEM BEHAV **25**(1) 161–170, 1986.—Locomotor activity was measured by photobeam interruptions, and metabolic rate by the production of CO_2 (as minute volume expired CO_2, or V_ECO_2) in mice. d-Amphetamine (0.3 to 10 mg/kg IP) increased locomotor activity in a dose-dependent manner while suppressing V_ECO_2 over the same 72-min test period, compared to saline-injected controls. This phenomenon of divergent effects on locomotor activity and metabolic rate required central stimulation, as neither ammonium sulfate nor p-hydroxyamphetamine suppressed V_ECO_2. Oxygen consumption was also suppressed by d-amphetamine, indicating that the suppression of V_ECO_2 involved more than a change in respiratory quotient. When baseline activity rates were increased with running wheels, V_ECO_2 and activity were both suppressed by d-amphetamine; V_ECO_2 was suppressed by d-amphetamine more in exercising mice than in sedentary mice. Anorexigenic agents phenmetrazine, aminoxaphen, and fenfluramine, when administered in doses equimolar to maximally effective doses of d-amphetamine, did not consistently affect activity or V_ECO_2. Evidence for mediation of the V_ECO_2 response by corticosterone and endogenous opioid peptides was negative. Further work, with other mediators of the stress response, or with more complete dose-effect studies with anorexigenic compounds, may be necessary to explicate the mechanism of this counter-intuitive divergence of two measures of activity in mice.

d-Amphetamine Aminoxaphen Fenfluramine Phenmetrazine p-Hydroxyamphetamine
Naloxone ACTH Corticosterone Metabolic rate CO_2 production Oxygen consumption
Locomotor activity Mouse

TWO prominent features of the psychopharmacology of amphetamine in rodents are its psychomotor stimulant effects [7, 23, 42] and its ability to suppress appetite [7,9]. Less well known are its effects on body temperature and metabolic rate. At room temperature, high doses (ca. 15 mg/kg) of d-amphetamine induce hyperthermia in rats [16], mice [31], rabbits [48], and cats [1]. In rats, 15 mg/kg d-amphetamine produces an ambient temperature-dependent biphasic response in body temperature: hyperthermia is induced at ambient temperatures of 20–37°C, and hypothermia at ambient temperatures of 4–15°C [45, 54, 58]. By contrast, low doses of the drug (ca. 1 mg/kg) have been shown to induce hypothermia at room temperature in rats [24] and in mice [32].

This amphetamine-induced hypothermia in rats appears to be mediated centrally [32] by dopaminergic pathways [59, 60, 61] in which endogenous opioid peptides may play a role [55,62]. In contrast, the hyperthermic effects of higher d-amphetamine doses appear to reflect peripheral sympathetic stimulation [18].

However, the role of changes in metabolic rate in these phenomena is not clear. Data relating amphetamine to metabolic rate are scarce and rarely derived from studies focused on this question. For example, parallel increases in locomotor activity and O_2 consumption have been reported in rats after d,l-amphetamine [36,51]; end-tidal CO_2 concentrations in humans were suppressed by d-amphetamine in a study of the analgesic effects of combined morphine and amphetamine [34]; and O_2 consumption was increased by amphetamine in rats made experimentally uremic and acidotic [21]. Finally, Yehuda and Kahn [56] were unable to show consistent changes in O_2 consumption, CO_2 production, and activity after 15 mg/kg d-amphetamine given to restrained rats, despite large concurrent changes in rectal temperature.

We previously showed that metabolic rate, as indexed by measurement of CO_2 production (as minute volume expired CO_2, V_ECO_2), was a useful index of overall activity in mice [3], and proved to be sensitive to inhalation of toluene vapor [4]. I now report that low doses of d-amphetamine reliably decrease V_ECO_2 while simultaneously increasing locomotor

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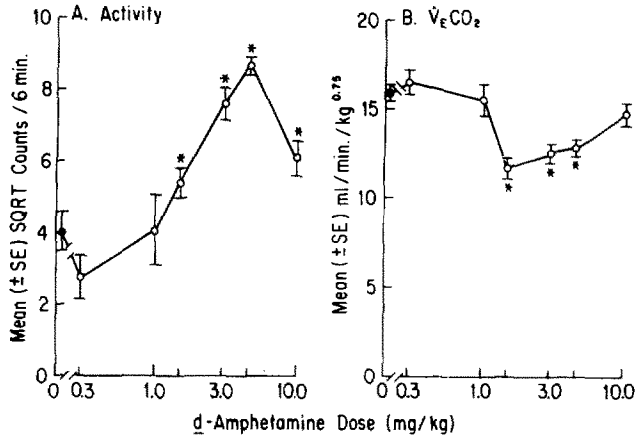

FIG. 1. Dose-effect functions for the effects of d-amphetamine on (A) locomotor activity and (B) V_ECO_2, averaged across the 72-min tests, showing all tested doses from 0.3 to 10 mg/kg. Asterisks indicated points which differ significantly from saline control.

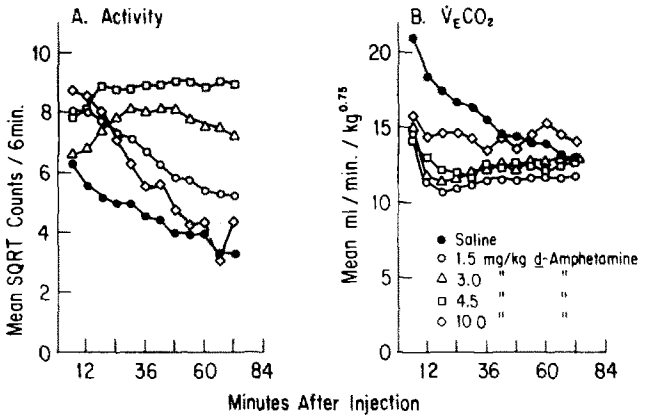

FIG. 2. Time course of the effects of d-amphetamine at effective doses of 1.5 to 10 mg/kg on (A) locomotor activity and (B) V_ECO_2. The responses to all doses shown were significant at $p<0.05$ across all time intervals except at 10 mg/kg, at which dose both measures returned to control levels during the 72-minute test.

activity in mice, and provide evidence against several possible mechanisms for the effect.

EXPERIMENT I: EFFECTS OF d-AMPHETAMINE ON V_ECO_2 AND ACTIVITY

METHOD

Subjects

Adult male C57BL/6J mice (Jackson Labs, Bar Harbor, ME), 20–40 g in weight, were housed in groups of four in acrylic cages (13 cm W × 28 cm L × 17 cm H) on pine chip bedding (Beta Chip, Northwestern Products, Warrensburg, NY). Housing rooms provided a 12 hr:12 hr light:dark cycle with light onset at 6 a.m., ventilation with a one-pass air supply at 12–15 air changes/hr, a temperature of 27±1°C and relative humidity between 45 and 65 percent. Rodent lab chow (Ralston Purina, St. Louis, MO) and water were available ad lib. Animal care practices conformed to standards promulgated by NIH [37]. Apparatus has been described in detail elsewhere [3]. Briefly, it consisted of eight mouse chambers in an isolation unit, each with an infrared photobeam (Model 1100, Autotron, Danville, IL) to detect locomotor activity. CO_2 concentrations were measured by two infrared CO_2 analyzers (LIRA Model 303, Mine Safety Appliances, Pittsburgh, PA) assorted plumbing, and two integrating chart recorders (Model 252A, Linear Instruments, Reno, NV) interfaced to a PDP8/a computer (PDP8/a, Digital Equipment, Maynard, MA) with SKED system (State Systems, Kalamazoo, MI). Two parallel channels for gas analysis permitted simultaneous measurement of CO_2 concentrations from two chambers; a time-sampling procedure was thus used, in which each chamber was sampled for 1.5 min during each 6-min sampling cycle. Gas flow and pressure were maintained by vacuum pumps and critical orifices at 1.3 l/min and 4–6 cm H_2O vacuum, respectively.

Drug Administration

d-Amphetamine sulfate (Pennwalt Co., Rochester, NY) was dissolved in sterile saline at concentrations of 0, 0.075, 0.25, 0.38, 0.75, 1.13, and 2.50 mg/ml (as the salt) and injected IP in a dose volume of 0.10 ml/30 g body weight, yielding doses of 0, 0.3, 1.0, 1.5, 3.0, 4.5, and 10.0 mg/kg. Each dose was administered to a separate group of mice (n=8). Each mouse was captured, weighed, injected, and placed individually into its test chamber for 72 min without food or water during the light (inactive) phase of the light cycle. Data collection began as soon as the CO_2 concentrations in the first pair of chambers to be sampled had reached a plateau (about 1 min). The system was calibrated daily.

Locomotor Activity

Frequencies of photobeam interruptions by the mouse were counted by the computer and normalized by square root transformation [35] prior to analysis.

V_ECO_2

CO_2 concentrations in the outflowing air of each mouse chamber were integrated over periods of 1.5 min in each 6-min sampling cycle. Airstream CO_2 concentrations (ml/l) were converted to minute volume expired CO_2 (V_ECO_2, in ml/min) by multiplication with total airflow (l/min). These volumes were than normalized to the metabolic mass [27] of each animal (body weight in kg raised to the 0.75 power). See [3] for further details.

Statistical significance was determined by a 2-factor analysis of variance (BMDP2V [15]), with drug as a between-groups factor and repeated measures for time interval; the Huynh-Feldt correction for nonindependent observations was used for repeated measures. Post-hoc comparisons between groups were done by simple main effects tests within significant interactions using pooled error terms as recommended by Kirk [26] and Dunnett's procedure to compare individual treatment means with the control [35]. The criterion for statistical significance was $p<0.05$ experimentwise.

RESULTS

When averaged across the 72-min test period,

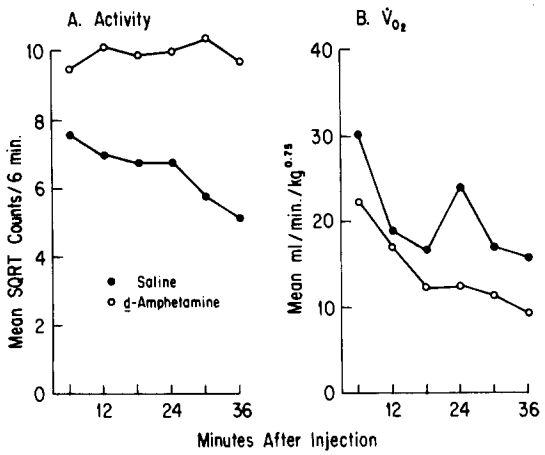

FIG. 3. Effects of 3 mg/kg d-amphetamine on (A) locomotor activity and (B) oxygen consumption (VO_2). The drug significantly affected both measures across all time intervals combined.

d-amphetamine dose-effect functions showed significant changes for locomotor activity, $F(5,42)=14.24$, $p<0.00001$, and for V_ECO_2, $F(5,42)=8.92$, $p<0.00001$. Locomotor activity was significantly increased at all doses above 1.0 mg/kg (Fig. 1A), with maximal stimulation at 4.5 mg/kg. By contrast, V_ECO_2 was significantly suppressed at doses of 1.5, 3.0, and 4.5 mg/kg (Fig. 1B). Maximal V_ECO_2 suppression occurred at 1.5 mg/kg and lessened with increasing dose: at 10 mg/kg, V_ECO_2 was suppressed only during the first half of the test session (see Fig. 2B below), thus reducing the overall effect of the dose.

The time courses of locomotor activity stimulation and V_ECO_2 suppression for effective doses of d-amphetamine only (1.5 to 10.0 mg/kg) are shown in Fig. 2. Analysis of the significant drug dose by interval interaction for locomotor activity, $F(55,462)=5.75$, $p<0.0001$, showed that the change in activity across time after injection depended upon amphetamine dose (Fig. 2A). At 1.5 mg/kg, a nonsignificant ($p<0.06$) increment in activity fell in parallel with the change in control activity. At 3.0 and 4.5 mg/kg, initial high levels of activity further increased and were maintained across time blocks, becoming significantly greater than control at 24 and 18 min postinjection, respectively. At 10 mg/kg, nonlocomotor stereotypical behavior, which does not produce photobeam breaks, overshadowed locomotor stimulation producing a decline in apparent activity to nonsignificant levels 24 min postinjection. In contrast, the drug dose by interval interaction for V_ECO_2, $F(55,462)=6.03$, $p<0.0001$, showed that V_ECO_2 suppression occurred rapidly—within 12 min postinjection—and returned to baseline during the next hour (Fig. 2B).

DISCUSSION

The amphetamine-induced changes in photobeam breaks are entirely consistent with numerous reports of the effects of this compound on activity levels in rodents, with low doses increasing locomotor activity and high doses inducing stereotypic behaviors which do not produce beam interruptions [7,42]. However, parallel changes in V_ECO_2 were not observed: no dose of amphetamine was found which would increase V_ECO_2, and the reductions in V_ECO_2 observed exhibited a dose-dependency and time course different from that for locomotor activity (Figs. 1 and 2). Nevertheless, considerable temporal overlap (12–36 min) existed, during which time locomotor stimulation and V_ECO_2 suppression occurred simultaneously.

The divergent effects of d-amphetamine on these two measures indicates that the normal correlation between locomotor activity and metabolic rate (+0.7 in these animals [3]) is disrupted by d-amphetamine. This finding contrasts with predictable effects on V_ECO_2 of diurnal cycles, peripheral sympathetic stimulation, fasting, and pentobarbital previously reported [3]. On the other hand, the pattern closely resembles that obtained from mice inhaling toluene vapor [4], and is consistent with reports of hypothermia in rodents after low doses of d-amphetamine [24,32].

The dose-effect functions for d-amphetamine on locomotor activity and metabolic rate (Figs. 1 and 2) indicate that the two responses are equally sensitive to the drug. Neither effect was observed below 1.5 mg/kg, above which a biphasic curve was obtained for both measures. In contrast to the inverted-U function for locomotor activity, however, the V_ECO_2 dose-effect function deflected sharply between 1.0 and 1.5 mg/kg, and the suppression of V_ECO_2 diminished progressively with increasing dose thereafter (Fig. 2). This function suggests that an all-or-none V_ECO_2 response to d-amphetamine was triggered at about 1.5 mg/kg, and that a second effect began to appear as the dose was increased. This second effect may be related to the hyperthermia induced by high doses of d-amphetamine (e.g. [1, 16, 45, 58]). Such doses may increase metabolic rate via peripheral sympathetic stimulation, as with epinephrine injection or exposure to cold air [3].

EXPERIMENT II: EFFECTS OF d-AMPHETAMINE ON VO_2 AND ACTIVITY

Experiment I demonstrated that amphetamine suppressed V_ECO_2 in mice. However, CO_2 production reflects metabolic rate precisely only when oxidative metabolism utilizes only carbohydrate [53]. When protein and fat are oxidized, O_2 consumption exceeds CO_2 production by a ratio known as the respiratory quotient (RQ). Thus, a drop in V_ECO_2 may reflect either a reduction of metabolic rate per se, or a change in substrate oxidation, or both.

Because d-amphetamine affects release of free fatty acids from adipose tissue [11,40], the increase in circulating free fatty acids may shift the substrate of oxidative metabolism toward fat and away from carbohydrate with a consequent reduction in the RQ. Under these conditions V_ECO_2 would be suppressed without a change in metabolic rate or consumption of O_2. Experiment II was designed to determine the effect of d-amphetamine on O_2 consumption (VO_2) in mice.

METHOD

Subjects were 4 male C57BL mice of similar age and weight to those used in Experiment I. Each animal was injected IP with saline or 3.0 mg/kg d-amphetamine sulfate (as in Experiment I) on alternate days. VO_2 was measured as described below for six 6-min blocks beginning 10 min after injection.

Oxygen consumption was measured using a modification of the method of Watts and Gourley [52]. A single mouse was placed in a stoppered 1-quart glass jar placed on its side in a

water bath maintained at 25°C. The jar contained a galvanized mesh floor which separated the mouse from a layer of sodium hydroxide (Ascarite, A. H. Thomas, Philadelphia, PA) on the bottom side of the jar. A 5-ml burette was introduced into a single opening in the stopper, thus permitting air to flow into the jar. A copper sponge was placed between the mouse and the inner opening of the burette to act as a heat sink and to prevent the mouse from breathing directly into the burette. To quantify locomotor activity, a single infrared photobeam bisected the short dimension of the jar.

The CO_2 exhaled by the mouse was absorbed by the sodium hydroxide, and inhaled O_2 not metabolized directly to CO_2 was retained by the mouse: air was thus pulled into the jar at a rate equal to the rate at which O_2 was withdrawn from the air by the mouse. This rate was determined by introducing a soap bubble into the burette and measuring with a stopwatch the time necessary for the bubble to travel 1.0 ml down the burette. To account for absorption of exhaled water vapor by the ascarite, each mouse was retested following injection of saline, using silica gel in place of ascarite. The air flow due to water absorption was then subtracted from the total flow, previously determined with ascarite, to yield a net flow due to O_2 consumption. Finally, these flows were converted to minute volume O_2 (VO_2) and divided by body weight to the 0.75 power to correct for differences in metabolic mass.

Statistical analysis utilized a two-way repeated measures analysis of variance [15]. Post-hoc analysis of significant interactions used alpha-corrected multiple t-tests to compare treatment means with control at each time interval.

RESULTS AND DISCUSSION

Oxygen consumption was suppressed by d-amphetamine (Fig. 3B) in a manner comparable to that seen for V_ECO_2, despite increased locomotor activity (Fig. 3A). The drug's main effect was significant for both activity, $F(1,3)=26.12$, $p<0.02$, and for VO_2, $F(1,3)=25.74$, $p<0.02$.

The drug-by-intervals interaction for activity was significant, $F(5,15)=3.29$, $p<0.04$, and followup t-tests indicated that d-amphetamine-treated mice were more active than controls in intervals 5 and 6. This interaction for VO_2 was not significant. Thus, VO_2 was also suppressed by d-amphetamine as was V_ECO_2 in Experiment I. The suppression of V_ECO_2 therefore did not simply reflect a d-amphetamine-induced change in the RQ.

EXPERIMENT III: EFFECTS OF p-HYDROXYAMPHETAMINE ON V_ECO_2 AND ACTIVITY

The metabolic response to d-amphetamine may be mediated directly by its action on the CNS or by way of its peripheral pressor effects. To determine whether the effects of d-amphetamine were mediated by central or peripheral effects of the drug, the d-amphetamine analog p-hydroxyamphetamine, which does not cross the blood-brain barrier [19], was used.

METHOD

Subjects were 16 male C57BL mice similar in age and weight to those used in Experiments I and II. Apparatus was identical to that in Experiment I. Procedures followed those of Experiment I, except that saline vehicle or p-hydroxyamphetamine at 8.1, 16.2, and 48 μmole/kg (equivalent on a molar basis to 1.5, 3, and 10 mg/kg d-amphetamine) were administered to all mice on different days in a counterbalanced order. Statistical procedures followed those of Experiment II.

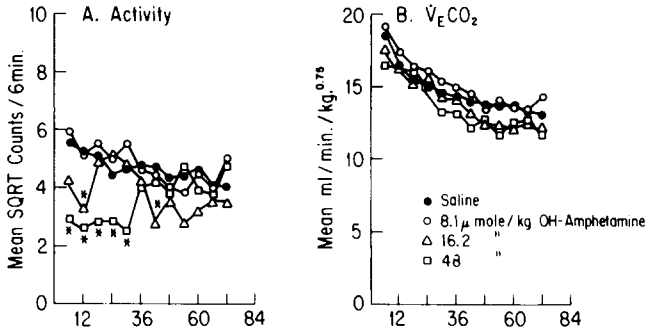

FIG. 4. Effects of p-hydroxyamphetamine on (A) locomotor activity and (B) V_ECO_2. Doses of 8.1, 16.2, and 48 μmole/kg are the molar equivalents to doses of d-amphetamine at 1.5, 3, and 10 mg/kg. Asterisks as in Fig. 1.

RESULTS

Activity was significantly suppressed (Fig. 4A) by p-hydroxyamphetamine, $F(1,15)=12.47$, $p<0.03$; this suppression recovered with time after drug administration [drug by time interval interaction, $F(11,165)=2.52$, $p<0.05$]. Analysis of this interaction also showed that 8.1 μmole/kg p-hydroxyamphetamine was without effect; that 16.2 μmole/kg suppressed activity at 12 and 42 min postinjection; and that 48 μmole/kg suppressed activity for the first 30 min postinjection (Fig. 4A). Despite this dose-related suppression of activity, no significant effect on V_ECO_2 was observed after any dose of the drug (Fig. 4B).

DISCUSSION

This result indicates that the effect of d-amphetamine on V_ECO_2 is mediated by a central effect of the drug, since p-hydroxyamphetamine lacks central action [19] and was ineffective in suppressing V_ECO_2 even at a dose level which lowered locomotor activity. As further evidence that direct CNS action by d-amphetamine is necessary for the V_ECO_2 suppression, injection of a solution of ammonium sulfate equimolar to 10 mg/kg d-amphetamine (48 μmole/kg) was without effect on either locomotor activity or V_ECO_2. Therefore, local irritation at the peritoneal injection site did not contribute to the behavioral effects observed.

EXPERIMENT IV: EFFECT OF INCREASED BASELINE ACTIVITY ON THE LOCOMOTOR AND METABOLIC EFFECTS OF AMPHETAMINE

Baseline locomotor activity levels 30 min after injection with saline were 2–6 counts/6 min interval (e.g., Fig. 2A). Even after the most highly activating dose of d-amphetamine, however, locomotor activity levels still did not approach the limits of physical activity for this animal. For example, 4.5 mg/kg d-amphetamine increased activity to 80 photobeam breaks/6 min (Fig. 2A), which required the animal to circle the 15 × 15 cm square floor of the chamber—breaking the photobeam twice per circuit—at just under 7 rpm. Since a 7 cm mouse makes the 45 cm circuit of the chamber in 6–7 body lengths, it need move only 40–50 body lengths per min (<1 per second) to maintain this fre-

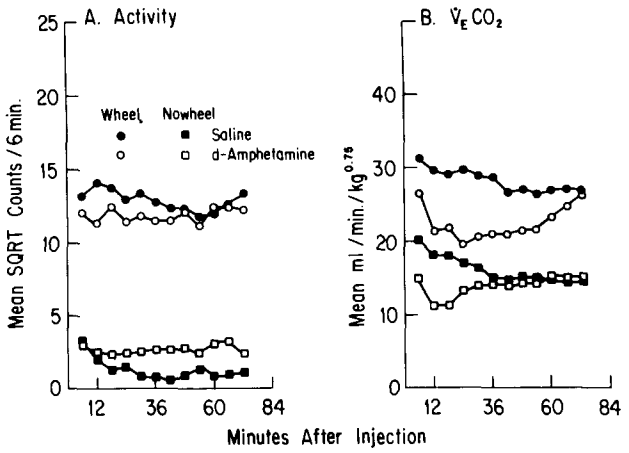

FIG. 5. Effects of d-amphetamine and the presence of running wheels on (A) locomotor activity and (B) V_ECO_2. Rate-dependent effects of amphetamine are evident in both measures: locomotor activity was increased at low baseline rates and suppressed at high baseline rates; V_ECO_2 was suppressed more at high rates than at low rates.

quency of photobeam breaks. Clearly, this effort cannot be severely taxing and could be maintained at somewhat reduced metabolic rates. Therefore, the effect of increasing baseline activity levels on the effect of d-amphetamine on locomotor activity and V_ECO_2 was investigated.

METHOD

Subjects were 16 female ICR mice (outbred). These animals were housed similarly but separately from the male C57BL previously used, and were of similar age and weight. They were used for this study because of their avid spontaneous running in wheels, in which they produced high levels of locomotor activity. Pilot studies with d-amphetamine injections under conditions identical to those used in Experiment I showed that V_ECO_2 effects equivalent to those at 3 mg/kg d-amphetamine in C57BL males were obtained at 1.0 mg/kg in this strain and sex.

Apparatus

Four of the 8 test chambers were modified to accommodate 15 cm dia. plastic hamster running wheels, and the photocells were realigned to count wheel revolutions. The remaining 4 chambers were unmodified.

Procedures followed those of Experiment I, except that half the mice were always placed in chambers with running wheels and the other half always in standard chambers. The behavior of mice running in wheels was compared to that of sedentary mice for 14 sessions prior to treatment with d-amphetamine. Half the mice in each test condition then received saline on one day and 1.0 mg/kg d-amphetamine on the next; the other half received the two treatments in the reverse order.

The data were analyzed in a 3-factor analysis of covariance [15], with the availability of the running wheel as a between-group factor and drug and time intervals as repeated measures. Baseline values for locomotor activity and V_ECO_2, obtained prior to drug treatment, were used as covariates. Post-hoc analysis followed that of Experiment I.

RESULTS

Wheel-running increased photobeam break frequencies by a factor of about 32 (Fig. 5A) and increased V_ECO_2 by about 75% (Fig. 5B). Also, d-amphetamine increased locomotor activity in sedentary mice without significantly affecting wheel-running (Fig. 5A). These results were as indicated by a significant interaction between the presence of the running wheel and d-amphetamine, $F(1,12)=8.77$, $p<0.02$, in the absence of an overall effect of the drug [main effect of drug, $F(1,12)=0.42$]. Simple main effects tests of the drug by wheel interaction showed that the drug increased the activity of sedentary mice, $F(1,12)=6.46$, $p<0.05$, but did not significantly change the activity of running mice, $F(1,12)=2.66$, $p>0.10$.

However, d-amphetamine suppressed the V_ECO_2 of both sedentary and running mice (Fig. 5B), as indicated by a significant overall effect of d-amphetamine, $F(1,12)=32.84$, $p<0.0001$. In addition, the suppression of V_ECO_2 by d-amphetamine was greater in running mice than in sedentary mice [drug by wheel interaction, $F(1,12)=6.42$, $p<0.03$].

DISCUSSION

Exercise in a running wheel increased both locomotor activity and V_ECO_2 to a far greater extent that a maximally stimulating dose of d-amphetamine (compare Figs. 2 and 5). d-Amphetamine-induced V_ECO_2 suppression coincident with increased locomotor activity in sedentary animals is thus not physiologically impossible. On the other hand, the fact that running wheel activity was not increased by d-amphetamine (Fig. 5A) suggests the existence of a locomotor activity ceiling when metabolic rate is suppressed (Fig. 5B).

Given this baseline difference, the effect of d-amphetamine on locomotor activity showed the classic rate-dependent effect, increasing activity only at low baseline levels [13,14]. The effect of the drug on V_ECO_2 was also rate-dependent: high rates of V_ECO_2 were more strongly suppressed by d-amphetamine than were low rates.

EXPERIMENT V: EFFECTS OF ANOREXIGENIC DRUGS ON LOCOMOTOR ACTIVITY AND V_ECO_2

Fasting has been shown to increase locomotor activity and to suppress V_ECO_2 in this preparation [3]. Fasting has also been shown to suppress metabolic rate [17] and peripheral sympathetic activity as assessed by cardiac norepinephrine turnover rates [29,30]. Thus, the possibility exists that drug-induced anorexia and fasting may have a common sympathetic correlate, and that the suppression of V_ECO_2 by d-amphetamine may be related to its ability to suppress appetite.

The locomotor stimulant effects of d-amphetamine may be pharmacologically differentiated from its anorexigenic effects [50] and separate CNS receptors for locomotor and anorexigenic effects of d-amphetamine have been found [39]. This evidence is consistent with the present effects of d-amphetamine on activity and V_ECO_2, since it provides a means by which independent and possibly divergent effects of the drug on locomotor and metabolic activity may be simultaneously produced.

To test the hypothesis that the suppression of V_ECO_2 induced by d-amphetamine is related to the anorexigenic properties of the drug, the anorexigens aminoxaphen, phenmetrazine, and fenfluramine were administered in doses equimolar to doses of d-amphetamine effective in increasing loco-

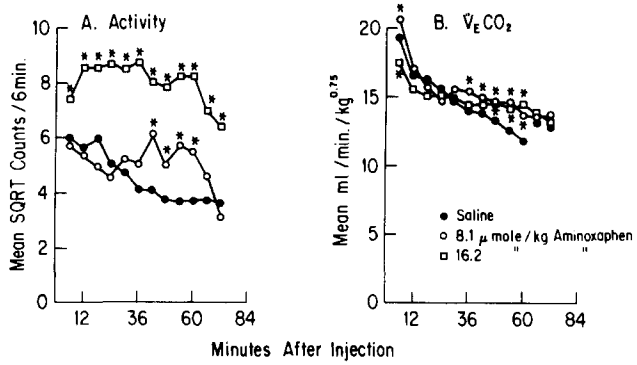

FIG. 6. Effects of aminoxaphen at 8.1 and 16.2 μmole/kg on (A) activity and (B) V_ECO_2. Asterisks as in Fig. 1.

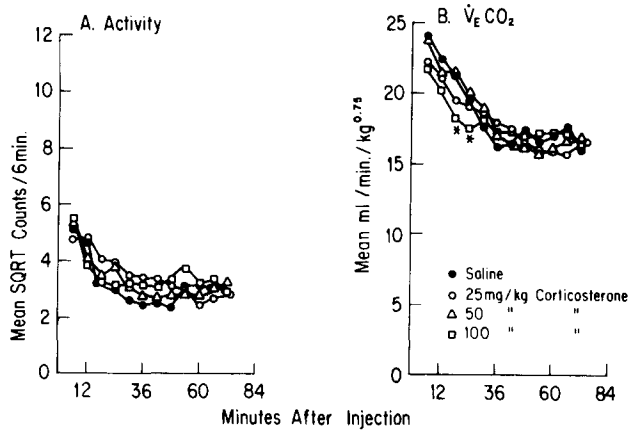

FIG. 7. Effects of corticosterone, 25, 50, and 100 mg/kg, on (A) locomotor activity and (B) V_ECO_2. Asterisks as in Fig. 1.

motor activity and suppressing V_ECO_2. On a molar basis, fenfluramine and aminoxaphen are more potent anorexigens than d-amphetamine in the rat; phenmetrazine and hydroxyamphetamine are less potent [9].

METHOD

Subjects were 16 male C57BL mice as in Experiment I. Apparatus was identical, and procedures similar to those of Experiment III, except for the compounds administered. Phenmetrazine hydrochloride (Boehringer Ingelheim, Ltd., Ridgefield, CT), aminoxaphen (MacNeil Pharmaceuticals, Spring House, PA), and fenfluramine hydrochloride (A. H. Robins, Richmond, VA) were each administered to each mouse at doses of 0 (saline control), 8.1, and 16.2 μmole/kg (equivalent to doses of 1.5 and 3.0 mg d-amphetamine per kg body weight). On any given test day, half the mice were injected IP with saline and the other half with one dose of one of the drugs. No mouse received drug injections more than twice per week. For statistical analysis, saline scores were averaged and compared in a 2-factor analysis of variance [15] with dose and time intervals as repeated measures. Significant interactions between the independent variables were further analyzed as in Experiment II.

RESULTS

Aminoxaphen increased activity in a dose-related manner (Fig. 6A); at 8.1 μmole/kg, activity was elevated 42–60 min after injection; at 16.2 μmole/kg, activity was increased at all intervals [drug main effect, $F(2,14)=13.06$, $p<0.0006$; drug by intervals interaction, $F(22,154)=2.32$, $p<0.01$]. V_ECO_2 was increased at 6 and 36–60 minutes after injection of 8.1 μmole/kg (Fig. 6B). After 16.2 μmole/kg, V_ECO_2 was suppressed for 6 minutes in a manner similar to that following d-amphetamine (Fig. 6B) but was then increased from 48–60 minutes postinjection [drug by intervals interaction, $F(22,154)=4.12$, $p<0.0001$].

Neither phenmetrazine nor fenfluramine had any significant effect on either measure at either dose.

DISCUSSION

Of these compounds, only aminoxaphen at 16.2 μmole/kg produced a d-amphetamine-like response. A comparision of Figs. 6A and 2A shows that this dose of aminoxaphen stimulated locomotor activity to a degree equal to 4.5 mg/kg d-amphetamine. By contrast, its suppression of V_ECO_2 was very small and brief compared to that produced by 4.5 mg/kg d-amphetamine (cf. Figs. 6B and 2B). Since aminoxaphen is a more potent anorexigen than d-amphetamine [9], the hypothesis that d-amphetamine's anorexigenic action suppresses V_ECO_2 predicts that aminoxaphen should suppress V_ECO_2 more than d-amphetamine, not less. It is therefore difficult to ascribe the suppression of V_ECO_2 by d-amphetamine to its anorexigenic action. It may nevertheless be possible to mimic d-amphetamine-like responses with appropriate doses of other anorexigens; however, complete ED_{50} studies were not done here. Such ED_{50} values would also permit correlative analysis of receptor binding with anorexigenic ED_{50} as performed by Paul *et al.* [39]. Such an analysis could thereby provide insight regarding the mechanism of V_ECO_2 suppression at the receptor level.

STUDIES INVOLVING PITUITARY-ADRENAL HORMONES

Unpublished evidence from this laboratory suggested that mice subjected to stress—e.g., physical restraint or injury, or drug overdose—showed reduced metabolic rate for a period of several minutes to hours after application of the stressor. Intoxication with inhaled toluene also suppressed metabolic rate while simultaneously increasing locomotor activity [4]. These observations suggested that the suppression of metabolic rate may be a component of the general adaptation syndrome of Selye [47] and may thus involve pituitary and adrenal hormones.

d-Amphetamine exerts a well-described increase in adrenal glucocorticoid secretion in rats [28]. Thus, the d-amphetamine-induced suppression of V_ECO_2 may be mediated by the activation of the pituitary-adrenal axis and and the release of corticosterone into the bloodstream.

Three experiments were conducted to test this hypothesis. The first and second involved direct administration of corticosterone and adrenocorticotrophic hormone (ACTH); support for the hypothesis would be gained by suppression of V_ECO_2 by both treatments. In the third experiment, the time courses of plasma corticosterone rise, activity increase, and V_ECO_2 suppression were determined after 3.0 mg/kg d-amphetamine. In addition, interanimal correlations be-

tween changes in plasma corticosterone concentration (plasma [CS]) and V_ECO_2 were calculated. A significant negative correlation between the change in plasma [CS] and the change in V_ECO_2 would provide further support for the hypothesis of corticoid mediation of V_ECO_2 suppression.

EXPERIMENT VI: EFFECTS OF INJECTED CORTICOSTERONE

METHOD

Subjects were 16 male ICR mice housed and maintained as in Experiment I; apparatus was identical to that in Experiment I. Each mouse was injected with 0, 25, 50 and 100 mg corticosterone (Sigma Chemicals, St. Louis, MO) per kg body weight on different days. The order of dosing was counterbalanced across subjects. Corticosterone was suspended by sonication in sterile saline and injected IP in a volume of 0.10 ml immediately prior to testing. Data were analyzed in a 2-factor repeated measures analysis of variance [15], as in Experiment III.

RESULTS AND DISCUSSION

Injected corticosterone had no consistent effect on activity (Fig. 7A). V_ECO_2 was suppressed slightly 18–24 minutes after 100 mg/kg corticosterone (Fig. 7B), as indicated by a significant dose by interval interaction, $F(33,495)=1.79$, $p<0.05$, and followup post-hoc tests [26]. If corticosterone mediates the d-amphetamine-induced suppression of V_ECO_2, one would expect direct injection of the hormone to have at least as great an effect as the drug. Since this was not evident, little support for the hypothesis was gained from this study.

EXPERIMENT VII: EFFECTS OF INJECTED ACTH

The minimal effect of corticosterone on V_ECO_2 may be due to insufficient levels of the hormone reaching the target organ(s). Administration of ACTH at a dose calculated to yield a maximal adrenal response should generate plasma corticosterone levels no lower than those induced by d-amphetamine injection, and thus suppress V_ECO_2 if the response is mediated by this hormone. A maximal adrenal response occurs with plasma ACTH concentrations of 300 μU/ml [49]; given a plasma volume of 1 ml in a 30-g mouse, this concentration of ACTH can be achieved with an IV bolus of 300 μU ACTH.

METHOD

Subjects and apparatus were identical to those in Experiment VI. Each of the 16 mice was injected via the tail vein on alternate days with saline or 300 μU ACTH (Sigma Chemicals, St. Louis, MO) in a volume of 0.10 ml. Data were analyzed as in Experiment II.

RESULTS AND DISCUSSION

Intravenous ACTH did not affect activity or V_ECO_2. It therefore seems unlikely that the d-amphetamine-induced suppression of V_ECO_2 is mediated by the pituitary-adrenal axis.

EXPERIMENT VIII: CONCURRENT EFFECTS OF d-AMPHETAMINE ON V_ECO_2 AND PLASMA CORTICOSTERONE

Direct stimulation of the pituitary-adrenal axis (Experiment VII) did not suppress V_ECO_2 similarly to injection of d-amphetamine. Nevertheless, the potential interactions among the hormones and d-amphetamine may shed light upon the mechanism of the d-amphetamine-induced suppression of V_ECO_2. Therefore, plasma [CS] was determined after saline or d-amphetamine injection to assess the covariation of the changes in V_ECO_2 and plasma [CS] after d-amphetamine.

TABLE 1

RELATIONSHIP BETWEEN PLASMA CORTICOSTERONE CONCENTRATION ([CS]) and V_ECO_2 RESPONSES AFTER 3 mg/kg d-AMPHETAMINE AT 18 AND 72 MINUTES POSTINJECTION

		[CS], ng/ml		V_ECO_2, ml/min/kg$^{0.75}$	
		Time after injection, min			
Treatment		18	72	18	72
Saline	Mean	73.5	82.8	22.76	20.32
	S.E.	22.7	14.7	1.12	1.51
d-Amphetamine	Mean	195.3*	119.5	16.57*	16.48
	S.E.	29.3	36.0	0.74	0.99

*Significantly ($p<0.05$) different from saline score.

METHOD

Subjects and apparatus were identical to Experiment VI. Each mouse was injected with saline or 3.0 mg/kg d-amphetamine on alternate days in a counterbalanced order. Half the mice (n=8) were removed from the test chambers after 18 minutes and bled via the tail for analysis of CS; the other half (n=8) were removed after 72 minutes for blood drawing. Difference scores reflecting the change in V_ECO_2 and plasma [CS] from saline to d-amphetamine treatment were used to evaluate the temporal relationships between the variables in response to the drug.

Blood samples (20 μl) were centrifuged and the plasma saved. Plasma [CS] was determined by radioimmunoassay using a modification of the method of Keith *et al.* [25]. Modifications included the use of commercially available antibody to corticosterone (Miles Laboratories, Naperville, IL), sample extraction with anhydrous ethyl acetate (Mallinckrodt) in place of ethyl ether, and use of tetra-tritiated corticosterone ([1,2,6,7-^{3}H]-corticosterone, NET-399, New England Nuclear, Boston, MA) in place of di-tritiated compound.

Statistical analysis included 2-factor ANOVAs with repeated measures across drug dose and time intervals for locomotor activity and V_ECO_2 for the 8 mice observed for the entire 72-min session. Corresponding data for the 8 mice removed after 18 min were not analyzed. In addition, plasma [CS] and V_ECO_2 were analyzed at 18 and 72 min postinjection in separate 2-factor ANOVAs comparing the two time intervals (between-group factor) with the two levels of drug treatment (repeated measure). Finally, the change in V_ECO_2 (drug minus saline) was regressed on the change in plasma [CS] (drug minus saline) across subjects (n=8 at each time interval) to determine the degree of correlation between the two measures at the two time intervals after injection of d-amphetamine.

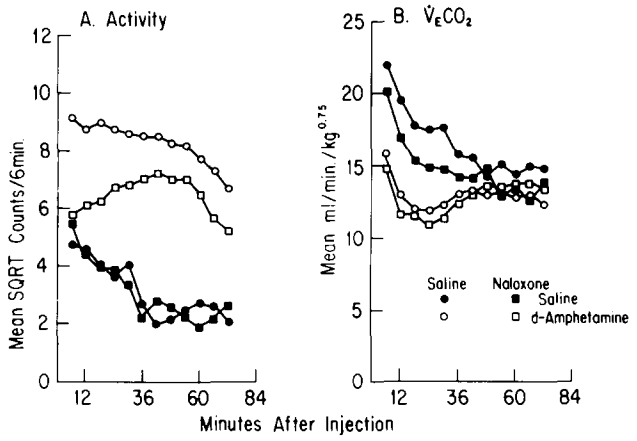

FIG. 8. Effects of naloxone on d-amphetamine-induced stimulation of (A) locomotor activity and (B) suppression of V_ECO_2.

RESULTS AND DISCUSSION

As before, 3 mg/kg d-amphetamine increased activity, $F(1,7)=8.13$, $p<0.03$, and suppressed V_ECO_2, $F(1,7)=21.25$, $p<0.003$. In addition, a significant d-amphetamine by intervals interaction, $F(11,77)=4.54$, $p<0.0002$, indicated that the suppression of V_ECO_2 by d-amphetamine was reversed during the 72-min test (Table 1). Plasma [CS] was significantly increased by d-amphetamine, $F(1,14)=6.49$, $p<0.03$, and declined between 18 and 72 min after injection (Table 1), thus following a time course similar to that of V_ECO_2.

However, it was not possible to demonstrate a significant correlation between the magnitude of [CS] rise and the magnitude of V_ECO_2 suppression in response to d-amphetamine across animals. In fact, at 18 minutes postinjection this correlation, though nonsignificant, $r(6)=0.66$, $p<0.10$, tended toward a postitive slope, suggesting that those animals whose [CS] rose the most in response to d-amphetamine showed the smallest suppression of V_ECO_2. At 72 minutes postinjection, the measures were uncorrelated and no slope could be estimated.

The results of these studies do not support the hypothesis that d-amphetamine-induced V_ECO_2 suppression is mediated by the pituitary-adrenal axis. The modest suppression in V_ECO_2 following corticosterone injection occurred only at the highest dose (Fig. 8B), and no response to ACTH in a dose sufficient to induce maximal adrenal stimulation was observed. Finally, the low correlation between V_ECO_2 suppression and plasma [CS] was in the wrong direction, suggesting not that corticosterone mediates the V_ECO_2 response, but that it may in fact inhibit it.

EXPERIMENT IX: INTERACTIONS WITH NALOXONE

In addition to stimulating the pituitary-adrenal axis, painful and injurious stimuli also activate endogenous opioid peptides in the CNS [20,33]. These peptides may affect metabolic functions, including thermoregulation [6,57] and ventilatory responses [10,41]. If such endogenous opioid activation mediates the suppression of V_ECO_2 in response to d-amphetamine, then blockade of opiate receptors should inhibit this suppression. It was thus hypothesized that pretreatment with naloxone, a specific opiate receptor blocking agent [19], would inhibit the suppression of V_ECO_2 induced by d-amphetamine.

METHOD

Subjects were 12 male C57BL mice as in Experiment I; apparatus was identical to that in Experiment I. Each mouse received two IP injections 30 minutes apart, followed immediately by testing for activity and V_ECO_2. Four conditions were employed: saline-saline; saline-3.0 mg/kg d-amphetamine; naloxone 30 mg/kg-saline; naloxone 30 mg/kg-d-amphetamine 3.0 mg/kg. This dose of naloxone has previously been used to study interactions with d-amphetamine in rats and mice [22]. Each mouse received the four treatments in a counterbalanced order on four different days. Statistical analysis utilized a 3-factor analysis of variance for each measure with amphetamine, naloxone, and time intervals as repeated measures. Post-hoc analyses followed those of Experiment III.

RESULTS AND DISCUSSION

In addition to a significant increase in activity due to d-amphetamine, $F(1,11)=95.89$, $p<0.00001$, and a significant decrease in activity due to naloxone, $F(1,11)=36.34$, $p<0.0001$, naloxone attenuated the stimulation of locomotor activity by d-amphetamine (Fig. 8A) as indicated by a significant naloxone by d-amphetamine interaction, $F(1,11)=22.17$, $p<0.0006$. By contrast, naloxone had no significant effect on V_ECO_2 overall [naloxone main effect, $F(1,11)=2.11$, $p=0.17$], nor did it attenuate the suppression of V_ECO_2 due to d-amphetamine (Fig. 8B) [d-amphetamine main effect, $F(1,11)=23.73$, $p<0.0005$; naloxone by d-amphetamine interaction $F(1,11)=3.49$, $p=0.09$].

Thus, this high dose of naloxone affected locomotor activity without affecting V_ECO_2, indicating that the effect of d-amphetamine on activity was antagonized by opiate receptor blockade, but that the effect of d-amphetamine on V_ECO_2 was not. These results are not consistent with the hypothesis that V_ECO_2 suppression by d-amphetamine is mediated by endogenous opioid peptides.

GENERAL DISCUSSION

These studies demonstrate that d-amphetamine suppresses metabolic rate in mice, an effect heretofore unsubstantiated for this compound in rodents. The fact that d-amphetamine can suppress metabolic rate while simultaneously increasing locomotor behavior indicates that the two measures of activity are somewhat independent and can be uncoupled at low levels of physical exertion. This uncoupling indicates that these two measures assess different kinds of activity in sedentary animals, and that metabolic rate does not always reflect degree of physical exertion. It is probably for such reasons that the two measures correlate positively but imperfectly in undrugged animals [3,38]. Similar reasoning justifies measuring V_ECO_2 to assess forms of metabolic activity not involved in physical exercise.

The relationship between d-amphetamine-induced suppression of V_ECO_2 and hypothermia is intriguing, but undocumented. Both appear to result from low doses of d-amphetamine [24,32] and both clearly require mediation by the CNS (Experiment III, [32]). The suppression of V_ECO_2 in the mouse (Experiment I) precedes temporally the drop in rectal temperature [32], suggesting that the change in temperature may result from the drop in metabolic rate. Effects of d-amphetamine at higher doses are complicated by the dependence of the direction of the effect on ambient temperature [45,58] and the overriding peripheral effects of the drug at that level [18].

Running wheel studies (Experiment IV) showed that baseline activity levels affect the response to d-amphetamine, but that V_ECO_2 suppression is robust in the face of this baseline change. Indeed, as shown in Fig. 5B, V_ECO_2 was suppressed more from the higher baseline than from the low baseline. This finding suggests that d-amphetamine may in fact limit running wheel activity by suppressing metabolic rate.

The mechanistic studies involving anorexigenic agents, pituitary-adrenal hormones, and naloxone did not clearly explicate any mechanism by which d-amphetamine suppresses metabolic rate. Opioid peptides whose receptors are blocked by naloxone do not seem to be involved, as naloxone did not reverse the d-amphetamine-induced suppression of V_ECO_2 (Fig. 8). It should be noted that naloxone has previously been shown to attenuate the locomotor stimulant effect of d-amphetamine in both rats [22] and mice [12].

The studies with pituitary-adrenal hormones were undertaken because of observations that mice consistently showed suppressed V_ECO_2 after application of a stressor. Effective stressors included chemical intoxication with toluene vapor [4], trimethyltin chloride [5] and a very high dose of phenmetrazine (270 μmole/kg); physical restraint or rotation [44]; or physical injury. The present data do not suggest that corticosterone and ACTH are involved in the suppression of V_ECO_2. However, it may still be the case that suppression of metabolic rate occurs as a component of the general adaptation syndrome of Selye [47], but it is not mediated by glucocorticoid secretion. In this regard, it should be noted that some steroid hormones can induce a deep and reversible anesthesia in rats [46], during which metabolic rate is probably very low.

The role of the anorexigenic effects of d-amphetamine in suppressing V_ECO_2 should be explored further. The fact that doses of p-hydroxyamphetamine, phenmetrazine, and fenfluramine equimolar to effective doses of d-amphetamine did not suppress V_ECO_2 does not rule out the possibility that higher doses would. Indeed, aminoxaphen, the most potent of these anorexigens [9], did slightly suppress V_ECO_2 at 16 μmole/kg (Fig. 6B), and phenmetrazine at the very high dose of 270 μmole/kg did also. Complete ED_{50} studies on these compounds could certainly shed light on the possible efficacy of anorexigenic compounds in affecting metabolic rate.

Finally, the mediation of d-amphetamine-induced changes in thermoregulation by central dopaminogic pathways [59–61] suggests the possibility that V_ECO_2 suppression may also involve these pathways. Studies with specific dopaminergic agonists and antagonists could provide evidence on this point rather quickly.

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