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Effects of *d*-Amphetamine and of β-Phenylethylamine on Fixed Interval Responding Maintained by Self-Regulated Lateral Hypothalamic Stimulation in Rats

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GREENSHAW, A. J., D. J. SANGER AND D. E. BLACKMAN. *Effects of* d-*amphetamine and of* β-*phenylethylamine on fixed-interval responding maintained by self-regulated lateral hypothalamic stimulation in rats.* PHARMACOL BIOCHEM BEHAV **23**(4) 519–523, 1985.—*d*-Amphetamine sulphate (0.25–4.0 mg/kg) and β-phenylethylamine hydrochloride (1.25–50 mg/kg) respectively were administered IP to two groups of rats (R1-R4 and R5-R8 respectively) responding on a fixed interval (FI) schedule (R1-R4 FI 60 sec, R5-R8 FI 30 sec) of electrical hypothalamic stimulation. The duration of each train of stimulation was controlled by the duration of each lever press that initiated stimulation. Under these conditions administration of *d*-amphetamine resulted in a marked increase in overall response rates on the FI 60 sec schedule. This effect was significant at 1, 2 and 4 mg/kg. *d*-Amphetamine had no significant effect on the duration of hypothalamic stimulation or on the duration of responses occurring during the FI. Administration of β-phenylethylamine resulted in a decrease in overall response rates on the FI 30 sec schedule. This effect was significant at 50 mg/kg. β-Phenylethylamine increased the duration of responses occurring during the FI, this effect being significant at 25 and 50 mg/kg, but had no significant effect on the duration of hypothalamic stimulation. These results indicate that the systemic effects of *d*-amphetamine on response rate, and of β-phenylethylamine on both response rate and response duration, are dissociable from changes in the self-regulated duration of lateral hypothalamic stimulation.

d-Amphetamine β-Phenylethylamine Fixed-interval Self-stimulation Lateral hypothalamus Response rate Response duration

d-AMPHETAMINE has received considerable attention as a compound which may increase the rewarding properties of electrical brain-stimulation [1, 20, 22, 24, 25, 28]. Despite numerous studies of the effects of *d*-amphetamine on behaviour maintained by electrical brain-stimulation it is evident, however, that the dissociation of the effects of amphetamine on reward and on other factors, such as the tendency to exhibit stereotyped or perseverative responding, remains problematic. For example, the two-lever auto-titration procedure developed by Stein and Ray [23], which is often used to assess reward-thresholds [20,28], does not allow a dissociation to be made between possible drug induced response perseveration [18] and a decrease in reinforcement threshold. It is notable that opposite effects have been reported after applications of *d*-amphetamine directly into the nucleus accumbens [17]; nevertheless without an analysis of intra-accumbens amphetamine effects on spatial aspects of performance, this perhaps remains a contentious issue. Furthermore, although reward enhancement has been clearly demonstrated under certain conditions [7], the effects of this drug on rate-independent measures of reinforcement are not always consistent with the view that reward is increased.

In recent studies Zacharko and Wishart [27] and Zacharko and Kokkinidis [26] have reported that administration of *d*-amphetamine resulted in an increase in the duration of electrical hypothalamic stimulation with a self-regulation procedure requiring a nosepoke or a lever-press response. This result is consistent with the effects of decreasing current intensity. In contrast to this report Atrens *et al.* [1] have reported amphetamine-induced decreases in the duration of lateral hypothalamic stimulation using a shuttle-box self-regulation procedure. Zacharko and Wishart [27] have suggested that the effects of *d*-amphetamine on self-stimulation behaviour may be partly determined by the form of the operant response, interpreting their results in terms of compatibility between the required operant response and the topography of stereotyped activity induced by *d*-amphetamine [16]. The present study was conducted to determine the effects of *d*-amphetamine and of the endogenous amine

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β-phenylethylamine [5] on the self-regulated duration of electrical hypothalamic stimulation under conditions which allow a relative comparison of effects of drugs on response duration per se and on the duration of electrical brain stimulation [12]. β-Phenylethylamine is structurally related to amphetamine (α-methylphenylethylamine) and it has been suggested that this endogenous amine may play a role in neural processes subserving reinforcement [21]. Thus it was of interest to compare the relative effects of these two compounds on a rate-independent correlate of reinforcement magnitude.

The effects of several doses of *d*-amphetamine (0.25–4.0 mg/kg) and of the β-phenylethylamine (1.25–50 mg/kg) were investigated when lever-pressing was maintained on a fixed-interval (FI) 60 sec or a FI 30 sec schedule of electrical hypothalamic stimulation respectively. On these schedules, as described previously [12], the duration of stimulation was under the animals' control. In this experiment the duration of responses that did not deliver stimulation was monitored in addition to the duration of hypothalamic stimulation. Thus under these conditions it was possible to analyse the effects of these compounds on a correlate of reinforcement magnitude (i.e., duration of stimulation; see [11,12]) and on the characteristics of the operant per se (i.e., rate of responding and duration of non-reinforced responses which occurred during the FI). As the duration of stimulation on this schedule is reliably affected by variation in reinforcement magnitude whereas response rate is not, this former measure is referred to as rate-independent. Furthermore, a notable feature of the FI schedule is that response rate may vary considerably without affecting reinforcement density. The effects of β-phenylethylamine on behaviour maintained by self-regulated electrical brain stimulation have not previously been reported.

METHOD

Eight male Wistar rats were used. The rats were approximately two months old and weighed 200–250 g at the time of surgery. They were individually housed with food and water constantly available in the home cages.

Surgery and Histology

The animals were implanted unilaterally with twisted bipolar electrodes (0.3 mm diameter) that were insulated, except for a cross-sectional area at the tip. Stereotaxic coordinates were AP +1.5, Lat +1.5, Vent −8.5 mm from skull surface and bregma, based on the atlas of König and Klippel [15]. Surgery was carried out under nembutal anaesthesia in aseptic conditions.

On completion of the experiment, the rats were killed and electrode placement verified. Following perfusion of the heart with 0.9% saline followed by 10% formalin, the brains were removed. The frozen brains were sectioned at 50 μ and the sections were mounted and stained with haematoxylin. All placements were located within the medial forebrain bundle at the level of the lateral hypothalamus. The A–P range of the placements corresponded to coronal sections A 4230 μ–A 5910 μ of the König and Klippel [15] atlas.

Apparatus

The experiment was carried out in two-lever operant test chambers (24 × 24 × 20 cm) each equipped with a house light and a light above each lever. Gold track slip rings were used to connect the subjects to a constant current stimulator. Current intensity was continuously monitored with an oscilloscope connected through a 10K resistor in series with the rat (intensities are expressed as r.m.s. values). Commodore PET microcomputers served to control the experiments and to record behavioural responses [9]. Response durations were recorded to the nearest 20 msec.

Procedure

The rats were trained to respond on the left lever under a FI schedule of electrical hypothalamic stimulation (60 Hz sine-wave 36–106 μA). The training procedure was as described by Greenshaw *et al.* [11]. The duration of stimulation was equal to the duration of each lever press that delivered reinforcement, to a maximum of 4 sec. Delivery of stimulation was paired with a light above the left lever which remained on for the duration of stimulation. Current intensity was adjusted so that the self-regulated duration of stimulation was in the range 0.5 to 1.0 sec for each animal. With rats R1–R4 daily sessions of FI 60 sec were terminated after the delivery of 45 reinforcers or after 3600 sec had elapsed, whichever occurred first. With rats R5–R8 daily sessions of FI 30 sec were terminated after the delivery of 60 reinforcers or after 2700 sec had elapsed, whichever occurred first. After at least 14 days when baselines of schedule-controlled responding appeared stable, the effects of *d*-amphetamine sulphate (0.25, 0.5, 1.0, 2.0, 4.0 mg/kg) were assessed with R1–R4 and R5–R8 were used for the analysis of effects of β-phenylethylamine hydrochloride (1.25, 12.5, 25.0, 50.0 mg/kg). As β-phenylethylamine is a relatively short-acting compound due to its rapid inactivation by MAO [5], a FI 30 sec schedule was used to maintain behaviour with R5-R8. With the FI 30 sec schedule the higher reinforcement density allowed a larger sample of reinforcements to be attained within a shorter time period. It was felt that this would improve the sensitivity of the analysis of the effects of β-phenylenthylamine on the present duration measures. With R5–R8, after completion of the β-phenylethylamine dose-response curve, a dose of 1.25 mg/kg of *d*-amphetamine sulphate was administered to provide a within-subject comparison of the effects of the two drugs. Both compounds were dissolved in 0.9% saline and were injected immediately prior to testing in an intraperitoneal volume of 1 ml/kg. Doses are expressed as the salt form and were administered in a mixed order with at least three control days intervening between consecutive doses. Equivalent volumes of 0.9% saline were administered on all non-drug days.

RESULTS

As described previously [11], the FI schedules maintained stable patterns of responding characterised by a pause immediately after the delivery of the reinforcer followed by an accelerating rate of responding over the remainder of the interval. The FI 30 sec schedule maintained higher overall response rates than the FI 60 sec schedule, 20.3 ± 6.2 (FI 30) and 7.1 ± 2.2 (FI 60) responses per minute mean ± SD.

Effects of d-*Amphetamine*

The effects of *d*-amphetamine on the duration of hypothalamic stimulation, the duration of non-reinforced responses (i.e., responses occurring during the FI) and overall

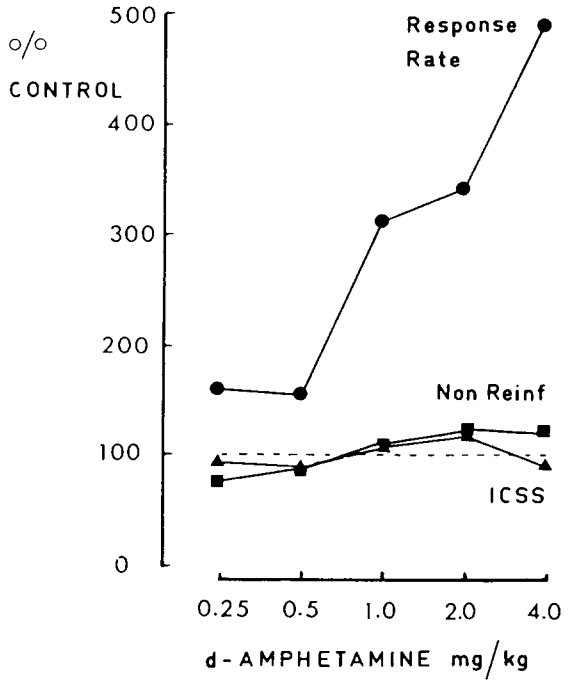

FIG. 1. The effects of several doses of *d*-amphetamine sulphate on overall response rate (●—●); the duration of non-reinforced responses (Non-Reinf) (■—■); and the duration of hypothalamic stimulation (ICSS) (▲—▲). Responding was maintained by a FI 60 sec schedule of hypothalamic stimulation.

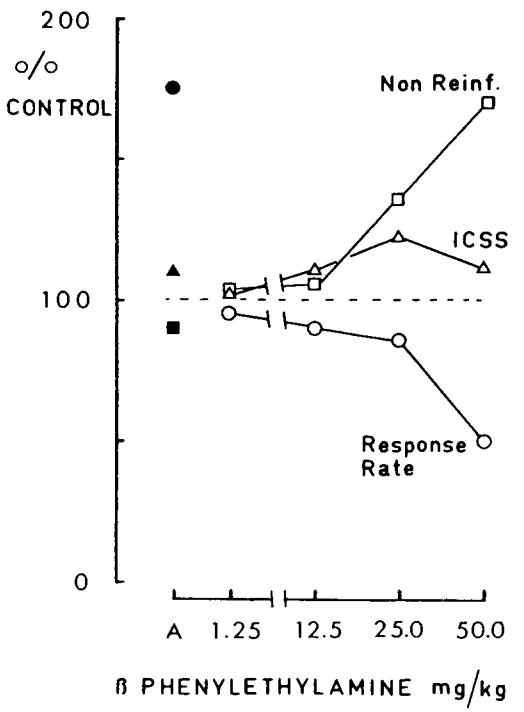

FIG. 2. The effects of several doses of β-phenylethylamine hydrochloride and of 1.25 mg/kg *d*-amphetamine sulphate (A) on overall response rate (○—○), the duration of non-reinforced responses (Non-Reinf) (□—□), and the duration of hypothalamic stimulation (ICSS) (△—△) maintained by a FI 30 sec schedule of hypothalamic stimulation. Filled symbols denoting amphetamine effects (A) correspond to open symbols for β-phenylethylamine.

response rates are displayed in Fig. 1. The data presented in this figure represent the mean percentage of baseline performance. These data were derived by expressing each animal's performance (R1–R4) on a drug administration day as a percentage of the value for the immediately preceding control day, the individual animal's percentage scores being averaged for each dose with each of the dependent variables. It is apparent from this figure that administration of *d*-amphetamine resulted in a marked increase in overall rates of responding on the FI 60 sec schedule. There were, however, no consistent or significant effects of this drug on the duration of hypothalamic stimulation or of non-reinforced responses. Analysis of variance (subjects × dose: repeated measures) revealed that the effects of amphetamine on response rate were statistically significant, $F(5,15) = 7.77$, $p<0.002$, whereas there were no significant changes in the duration of hypothalamic stimulation, $F(5,15) = 0.90$, $p>0.5$, or of non-reinforced responses, $F(5,15) = 1.57$, $p>0.2$. Multiple comparisons (Dunnett $\alpha = 0.05$) revealed that the rate-increasing effects of *d*-amphetamine were significant at 1.0, 2.0 and 4.0 mg/kg.

Effects of β-*Phenylethylamine*

The effects of β-phenylethylamine and of a 1.25 mg/kg dose of *d*-amphetamine on the duration of hypothalamic stimulation, the duration of non-reinforced responses and overall response rates are displayed in Fig. 2. The data presented in this figure represent the mean percentage of baseline performance for R5–R8 as described for Fig. 1.

Administration of β-phenylethylamine resulted in a decrease in overall response rates on the FI 30 sec schedule and an increase in the duration of non-reinforced responses; there was also a slight increase in the duration of hypothalamic stimulation. In contrast to these effects the administration of 1.25 mg/kg of *d*-amphetamine resulted in an increase in overall response rates but no change in the two response duration measures.

Statistical analysis of these data revealed that the β-phenylethylamine-induced changes in response rate, $F(4,12) = 21.67$, $p<0.001$, and in the duration of non-reinforced responses, $F(4,12) = 7.40$, $p<0.01$, were significant. There were, however, no statistically significant changes in the duration of hypothalamic stimulation, $F(4,12) = 2.02$, $P>0.2$. Multiple comparisons (Dunnett $\alpha = 0.05$) revealed that the rate depressant effects of β-phenylethylamine were significant at 50 mg/kg. The change in the duration of non-reinforced responses was due to a significant increase in this measure at 25.0 and 50.0 mg/kg of β-phenylethylamine. With respect to effects of *d*-amphetamine on FI 30 sec responding: Response rate was increased, $t(3) = 3.47$, $p<0.05$, whereas the duration of non-reinforced responses and of stimulation were unaffected, $t(3) = 1.06$ and $t(3) = 0.79$ respectively, $p>0.2$ in each case.

DISCUSSION

In the present experiment administration of *d*-amphetamine resulted in increased overall response rates maintained by a FI 60 sec schedule of electrical hypothalamic stimulation. These results are consistent with those of earlier reports of the effects of this drug on schedule controlled

responding maintained by electrical hypothalamic stimulation [2, 3, 14, 22].

Administration of *d*-amphetamine did not result in any consistent or significant changes in the duration of non-reinforced responses or in the duration of hypothalamic stimulation. These response duration data are consistent with those of a previous report describing the effects of administration of *d*-amphetamine on the duration of lever-presses on a tandem fixed-ratio, continuous reinforcement schedule, FR 24 (CRF, CRF, CRF, CRF), maintained by water delivery [6]. In this study Fowler *et al.* reported that administration of *d*-amphetamine (0.4 to 3.2 mg/kg) resulted in changes in peak response force and a shift in the distribution of inter-response times. However, administration of the drug did not result in any significant changes in response duration.

The lack of a consistent effect of *d*-amphetamine on the duration of self-regulated hypothalamic stimulation in the present study is inconsistent with earlier reports [1, 26, 27]. These earlier studies reported contrasting effects of the drug on this measure. Atrens and his colleagues [1], using a shuttle box procedure, reported reductions in the duration of lateral hypothalamic stimulation after administration of *d*-amphetamine (0.5 to 6.0 mg/kg), whereas Zacharko and Wishart [27] and Zacharko and Kokkinidis [26] reported increases in the duration of lateral hypothalamic stimulation (using a nose-poke response measure) after administration of the drug (1.0 to 5.0 mg/kg). These conflicting results illustrate that the effects of *d*-amphetamine may be dependent on the behavioural test procedure. In relation to this, Zacharko and Wishart [27] have suggested that the effects of *d*-amphetamine may be determined by the compatibility of the operant with the general effects of this drug on the topography of motor activity [16].

The rate-depressant effects of β-phenylethylamine on responding maintained by a FI 30 sec schedule observed in the present study are consistent with previous reports of effects of similar doses of this compound on operant responding maintained by hypothalamic stimulation [13] or by food delivery [8]. It has been proposed that β-phenylethylamine may increase the rewarding properties of hypothalamic stimulation [22]. In the present experiment, however, this compound increased the duration of non-reinforced responses without significantly altering the duration of hypothalamic stimulation.

As the FI 30 sec schedule maintained higher overall response rates than the FI 60 sec schedule, it is possible that the depressant effects of β-phenylethylamine may be dependent on baseline rate [19]. Nevertheless a stimulant effect of this compound on operant responding has never been reported. Indeed, by systematically varying the intensity of hypothalamic self-stimulation, Howard *et al.* [13] have demonstrated that over a wide range of response rates, β-phenylethylamine at various doses has a uniformly depressant effect on self-stimulation, in contrast to the effects of *d*-amphetamine. The Howard *et al.* study would, therefore, tend to exclude a rate-dependency interpretation of the present results. It is, however, notable that the effective range of doses of both *d*-amphetamine and of β-phenylethylamine in the present study are within the range of doses that induce characteristic syndromes of stereotyped behaviour [4]. In this regard it is apparent that prominent components of stereotyped responding induced by *d*-amphetamine (e.g., increased forward walking and rearing) may be more compatible with the coordinated act of lever pressing than the prominent components of stereotyped responding induced by β-phenylethylamine, e.g., forepaw padding and backward walking [10].

Previous studies with the present FI schedule have demonstrated that the duration of hypothalamic stimulation is inversely related to the intensity of hypothalamic stimulation [11,12] and that the duration of stimulation may be altered by drug treatment under the present conditions [12]. Response rate and the duration of non-reinforced responses are, however, not reliably altered by changes in current intensity [12]. The results of the present study indicate that the systemic effects of *d*-amphetamine on response rate and of β-phenylethylamine on both response rate and response duration may be independent of changes in the duration of self-regulated hypothalamic stimulation. Furthermore, together with previous reports [1, 26, 27] these data indicate that the systemic effects of amphetamine on the self-regulated duration of reinforcing hypothalamic stimulation may be dependent on the schedule that maintains behaviour.

In view of the relatively high effective doses in the present study, particularly for *d*-amphetamine, it may be the case that self-stimulation responses under strong schedule control (e.g., FI responding, as indicated by resistance to extinction: see [11]) may provide a less sensitive baseline with which to investigate effects of drugs on "reward processes" than schedules of self-stimulation which yield rapid extinction (e.g., CRF). This possibility underscores the need to assess effects of drugs on various aspects of the reinforcement process which may lead to a better understanding of schedule-dependent effects of drugs.

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