The effects of chronic administration of ceronapril on the partial reinforcement extinction effect and latent inhibition in rats

I. Weiner¹, R. Tarrasch¹, O. Hasson¹, R. Forian¹, A.D. Smith², J.N.P. Rawlins³ and J. Feldon¹

¹Department of Psychology, Tel-Aviv University, Ramat-Aviv, Tel-Aviv, Israel 69978, ²Department of Pharmacology, University of Oxford, Mansfield Road, Oxford OX1 3Q1, and ³Department of Experimental Psychology, University of Oxford, South Parks Road, Oxford OX1 3UD, UK

Correspondence to: I. Weiner at above address

Previous experiments showed that acute administration of the angiotensin converting enzyme (ACE) inhibitor, ceronapril, shares with neuroleptic drugs an ability to enhance latent inhibition (LI), which consists of retardation in conditioning to a stimulus as a consequence of its prior non-reinforced pre-exposure. Experiment 1 tested whether ceronapril would produce a neuroleptic-like effect in the partial reinforcement extinction effect (PREE) at one trial a day. The PREE refers to the increased resistance to extinction observed in animals trained on a partial reinforcement (PRF) schedule compared with those trained on a schedule of continuous reinforcement (CRF). Two groups of rats were trained to run in a straight alley. The CRF group received food reward on every trial. The PRF group was rewarded on a quasi-random 50% schedule. All animals were then tested in extinction in which no reward was given. Ceronapril at a dose of 0.05 mg/kg was administered in a 2 × 2 design, with drug or no drug in acquisition and drug or no drug in extinction. Rats receiving vehicle in acquisition showed a PREE, regardless of their drug treatment in extinction. In contrast, ceronapril administered in acquisition attenuated the PREE irrespective of drug treatment in extinction, by both increasing resistance to extinction in CRF animals and decreasing resistance to extinction in PRF animals. This pattern of results does not resemble that produced by neuroleptics. The PREE procedure necessitated repeated administration of ceronapril, whereas the previous demonstrations of neuroleptic-like enhancement of LI have been obtained with acute administration. Experiment 2 therefore tested the effects of chronic ceronapril administration on LI. Under these conditions, ceronapril abolished LI. The results are discussed in relation to the antipsychotic, anti-anxiety and cognitive-enhancing effects formerly attributed to ACE inhibitors.

Keywords: Ceronapril – Continuous reinforcement – Latent inhibition – Partial reinforcement – Partial reinforcement extinction effect – Pre-exposure – Rat

INTRODUCTION

It has been proposed that the consequences of elevated cholecystokinin (CCK) levels in the brain may resemble those of neuroleptic administration (Nair et al., 1985; Innis et al., 1986; Peselow et al., 1987). Angiotensin converting enzyme (ACE) inhibitors have been shown to retard the breakdown of central CCK (Rose et al., 1989). Since such a treatment would be expected to increase the functional levels of CCK in the brain and possibly potentiate and prolong its actions, we recently investigated the possible neuroleptic-like action of ceronapril, an ACE inhibitor that has already been found to have some central actions (Costall et al., 1989). The study used an animal model that simulates a cognitive dysfunction characteristic to certain forms of schizophrenia, namely attentional deficit. The model is based on the paradigm of latent inhibition (LI), in which non-reinforced pre-exposure to a stimulus retards conditioning to that stimulus when it is subsequently paired with a reinforcer (Lubow, 1973). For example, if an animal is pre-exposed to a series of tones, these tones lose their capability to enter into associations with other stimuli, such as shock, or responses such as shuttle avoidance. This decremental process is considered to reflect a process of learning to ignore, or to tune out, or not to attend to, irrelevant stimuli (Mackintosh, 1973, 1975, 1983; Moore, 1979; Lubow et al., 1981; Lubow, 1989).

LI is facilitated by neuroleptic drugs (Weiner and Feldon, 1987; Weiner et al., 1987b; Christison et al., 1988;
Feldon and Weiner, 1991a, c; Dunn et al., 1993), and this effect has been shown to be specific and selective for this class of drugs (Dunn et al., 1993). Our previous study (Weiner et al., 1992) evaluated the effects of 5, 50 and 500 µg ceronapril on LI. Only the 50 µg dose facilitated LI, and this enhancement effect was confirmed in a separate experiment.

Since this outcome suggested that ceronapril may possess some antipsychotic properties, the present study sought to extend the investigation of the parallels between the actions of neuroleptics and those of ceronapril by using an additional paradigm, the partial reinforcement extinction effect (PREE). The PREE consists of slower extinction of animals trained with partial reinforcement (PRF) compared with animals trained with continuous reinforcement (CRF). Administering haloperidol during extinction in this paradigm leads to decreased resistance to extinction (Feldon et al., 1988; Feldon and Weiner, 1991b). Haloperidol administration in acquisition leads to greater resistance to extinction in CRF animals (Feldon et al., 1988; Rubin, 1991), and either does not affect (Feldon et al., 1988) or further increases resistance to extinction of PRF animals (Rubin, 1991).

In order to permit a specific comparison to be made with an acute dose that was identified as effective in our LI experiments and in the light of our observation that the dose-response curve takes the form of an inverted U, we tested the effects of 50 µg ceronapril on the PREE. As in our previous work with haloperidol, ceronapril was administered in acquisition alone, in extinction alone, or in both stages of the PREE procedure. The results obtained did not resemble those found with neuroleptics. Since the PREE procedure necessitated repeated administration of ceronapril, whereas the neuroleptic-like enhancement of LI had been obtained with acute administration, Experiment 2 tested the effects of chronic ceronapril administration on LI. As in previous experiments, LI was assessed using a conditioned emotional response (CER) procedure in rats licking for water, consisting of three stages: preexposure, in which the to-be-conditioned stimulus (a tone) was repeatedly presented without being followed by reinforcement; conditioning, in which the preexposed stimulus was paired with reinforcement (a footshock); and test, in which LI was indexed by animals’ degree of suppression of licking during tone presentation.

METHODS

Subjects
A total of 176 male Wistar rats (Tel-Aviv University Medical School, Israel) approximately 4 months old were used. They were housed four to a cage under reversed cycle lighting. Animals used in Experiment 1 (n = 96) were fed for 1 h a day, commencing at least 1 h after the last animal had been run that day. Water was freely available. For Experiment 2, subjects (n = 80) were maintained on freely available food and water for 3 weeks after delivery. On the 22nd day animals were weighed and placed on a 23 h water restriction schedule which continued throughout the experiment. The animals were handled for 5 days starting on the 22nd day (see below); a chronic 21 day drug injection regime started on the 27th day (see below).

Apparatus
The apparatus for Experiment 1 consisted of a straight alley made of transparent Perspex with black rubber curtains covering the sides. The runway was 140 cm long, 15 cm wide and 35 cm high, with a startbox (20 cm long) and a goalkbox (20 cm long) separated by a run section (100 cm long). The floor consisted of a metal grid composed of equally spaced rods. The startbox door was made of transparent Plexiglas and opened vertically downwards. The door was operated by a solenoid controlled by a pushbutton. The goalkbox door was of metal and could be raised and lowered manually. Food pellets were placed in a recessed compartment 1 cm wide and 2.5 cm deep at the far side of the goalkbox. There were three light photobeams and photocells, the first one 2 cm beyond the startbox, the second 2 cm before the goal section and the third inside the goalkbox, which was interrupted when the rat contacted the food compartment. The photobeams operated three electronic timers, accurate to 0.01 s. The first timer timed the start section (from the opening of the start door to the first photobeam); the second timed the run section (from the second to the third photobeam) and the third, the goal section (from the second to the third photobeam). Prior to each trial, the goalkbox door was raised and, on rewarded trials, food was manually placed in the food compartment. Each reward consisted of 10.45 mg Campden Instruments food pellets. Once the animal interrupted the goalkbox photobeam, the goalkbox door was lowered. An IBM-PC computer was used for equipment programming and data recording.

The apparatus for Experiment 2 consisted of four Campden Instruments rodent test chambers (Model 410), each set in a ventilated sound-attenuating Campden Instruments chest (Model 412). A drinking bottle could be inserted into the chamber through a 0.5 cm diameter hole located in the centre of the left wall of the chamber, 2.5 cm above the grid floor. When the bottle was not present, the hole was covered by a metal lid. Licks were detected by a drinkometer circuit (Campden Instruments drinkometer Model 453). The preexposed, to-be-conditioned stimulus was a 2.8 kHz tone produced by a Sonalert module (Model SC 628). Shock was supplied by a Campden Instruments shock generator (Model 521/C) and shock scrambler (Model 521/S) set at 0.75 mA. Equipment programming and data recording were computer controlled.
Experiment 1

Following 1 week of food restriction, all animals were handled daily for 2 weeks and given 3 days of pretraining. On Day 1 of pretraining, animals were introduced into the alley in groups of four for 20 min with all alley doors open. On the second day, animals were placed in the alley in pairs for 10 min. On Day 3, each animal was placed individually in the alley for 5 min. On all days food pellets were available in the goalbox compartment and the experimenter ensured that all animals reached the goalbox and ate from the food compartment.

On the following day, acquisition began. One trial/day was administered for 15 days. On each day, the animal was placed in the start section and the three time measurements for the start, run and goal sections were recorded. The CRF groups received a reward on every trial throughout the 15 acquisition days. The PRF animals were rewarded on Days 1-12 on a quasi-random 50% schedule of RRRNNRRRNNRNNRNNR, where R is a rewarded trial and N is a non-rewarded trial. The experimenter ensured, on rewarded trials, that the animal consumed all the food pellets. There were no observable differences in consumption times between the drug-treated and vehicle animals. On Days 12-15 the PRF groups received reward on every trial (see Drug injections).

On Day 16, extinction started and continued for 30 days. In extinction, animals were run as in acquisition but no rewards were given. On non-rewarded trials during acquisition and during all extinction trials the rats were confined in the goalbox for 30 s.

Experimental design and drug treatment. The animals were assigned randomly to one of eight conditions (12 subjects in each condition) in a $2 \times 2$ factorial design. Half the rats received CRF training in acquisition, and half received PRF training; within these two reinforcement conditions the rats were given either ceronapril (CERON) or vehicle injections in acquisition. In extinction, these four groups were further subdivided into groups given CERON or vehicle treatments. The appropriate drug, either 0.05 mg/kg CERON dissolved in 1 ml saline or an equivalent volume of saline, was given i.p. 30 min prior to the daily trial. The last 3 days of acquisition were used for gradually tailing off the drug in the CERON-vehicle groups and gradually introducing the drug in the vehicle-CERON groups. The gradual introduction of the drug was based on Weiner et al. (1985) and the tailing off technique was modelled after Feldon and Gray's (1981a) Experiment 2. Both procedures served to prevent a drop in running speeds when animals were switched from drug to vehicle, or from vehicle to drug, as acquisition ended and extinction started. The CERON-vehicle groups were given CERON doses of 0.025 and 0.0125 mg/kg on Days 13 and 14 of acquisition, respectively, vehicle on Day 15 and vehicle throughout the 30 days of extinction. The vehicle-CERON groups were given CERON doses of 0.0125, 0.025 and 0.05 mg/kg on Days 13, 14 and 15 of acquisition, respectively, and 0.05 mg/kg CERON throughout extinction. The CERON-CERON groups received 0.05 mg/kg CERON throughout acquisition and extinction, while the vehicle-vehicle groups received saline throughout acquisition and extinction.

Statistical analyses. The data were transformed into reciprocals (1/x) to allow the use of analysis of variance. ANOVAs were performed for the acquisition and extinction phases. For each phase, start, run and goal data were analysed separately. The analysis of the acquisition data included two between-subjects factors: Reinforcement schedule (CRF and PRF) and Drug in acquisition (CERON and vehicle). There was one within-subjects factor: a repeated measurements factor comprising five blocks, each being the mean of three consecutive acquisition trials. The analysis of the extinction data included three between-subjects factors: Reinforcement schedule in acquisition, Drug in acquisition and Drug in extinction. The repeated measurements factor comprised 10 blocks, each being the means of three consecutive extinction trials. Both the acquisition and extinction analyses included trend analysis using the 2V program of the BMDP package. Two subjects (one from the VEH-VEH-PRF and one from CERON-VEH-PRF groups) were dropped from the experiment because they failed to acquire the running response during the first 3 days of acquisition. Thus, the final analysis was performed on 94 subjects.

Experiment 2

The animals were handled individually for about 2 min each day during Days 22-26 inclusive. The CER procedure started on Day 41. The animals were allocated to squads, each of four subjects, which were tested simultaneously except on the final test day. Each individual subject was trained and tested throughout in the same experimental chamber as follows.

Baseline: Days 41-45. On each of 5 days, the animals were placed in their experimental chambers and allowed to drink for 20 min.

Pre-exposure: Day 46. Each animal was placed in its experimental chamber, from which the bottle had been removed. The pre-exposed (PE) animals received 30 10 s tones with a variable inter-stimulus interval (ISI) with a mean of 30 s. The non-pre-exposed (NPE) animals were confined to the chamber for an identical period of time but did not receive the tone.
BEHAVIOURAL EFFECTS OF CERONAPRIL

Conditioning: Day 47. Each animal was placed in its experimental chamber, from which the bottle had been removed, and received two tone-shock pairings, given 5 min apart. Tone parameters were identical to those used in pre-exposure. The 0.75 mA, 1 s shock immediately followed tone termination. The first tone-shock pairing was given 5 min after the start of the session; the second pairing was 5 min later. After the second pairing, animals were left in the experimental chamber for an additional 5 min.

Rebaseline: Day 48. Each animal was given a drinking session identical to the baseline sessions. Latency to the first lick and the total number of licks during the session were recorded for each subject. Note that there were no drug injections on this day, nor on the following (test) day.

Test: Day 49. Each subject was individually placed in the chamber and allowed to drink from the bottle. When the subject completed 75 licks the tone was presented, and lasted 5 min. The following times were recorded: time to first lick, time to complete licks 1-50, time to complete licks 51-75 (pre-tone) and time to complete licks 76-100 (tone-on). The times to complete licks 76-100 were subjected to logarithmic transformation to ensure that the distribution of the data was suitable for parametric analysis of variance. In addition, the suppression of drinking in response to tone presentation was assessed by the number of licks during the presentation of the tone, recorded in 10 s segments.

Experimental design and drug treatment: The 80 animals were randomly assigned to four experimental groups in a 2 x 2 factorial design. The factors were Pre-exposure (0, 30) and Drug (vehicle, 0.05 mg/kg CERON). During Days 27-40 animals were given daily (between 09.00 and 10.00 h) i.p. injections of 0.05 mg/kg CERON dissolved in saline (1 ml/kg) or of saline (1 ml/kg). On Days 41-47 the injections were 30 min prior to the start of the daily session. Data from one subject were lost due to apparatus failure (from the NPE-vehicle group). Thus, the final analyses were performed on the data from 79 subjects.

RESULTS

Experiment 1

Acquisition. The results, expressed in mean running speeds (1/s) in the Start, Run and Goal sections of the alley, as a function of acquisition reinforcement schedule (continuous, CRF, or partial, PRF reinforcement) and acquisition drug condition (50 μg ceronapril, CER, or vehicle, VEH). The 1 S.E. bar represents one standard error derived from the ANOVA.

and Run [F(1,90) = 5.69, p < 0.02] sections. In addition, the main effect of Reinforcement approached significance in the Start [F(1,90) = 3.03, p < 0.09], and Goal [F(1,90) = 2.86, p < 0.10] sections, and the interaction of Reinforcement x Days approached significance in the

Run [F(4, 360) = 2.26, p = 0.06] and Goal [F(4, 360) = 2.23, p < 0.07] sections. There was no effect of drug condition during acquisition: neither the main effect nor any of the interactions attained significance.

Extinction. Figure 2 depicts extinction performance in the Goal section, which is representative of the results in the Start and Run sections. As can be seen the rats in the PRF groups extinguished the running response more slowly than the rats in the CRF groups. This PREE was clear in all three alley sections. The statistical analyses supported this conclusion as follows. In the Start section, there was a significant Reinforcement × Days interaction [F(9, 774) = 3.33, p < 0.005; quadratic component F(1, 86) = 5.29, p < 0.05]. In the Run section, the main effect of Reinforcement [F(1, 86) = 9.62, p < 0.005] and the Reinforcement × Days interaction [F(9, 774) = 7.62, p < 0.001; quadratic component F(1, 86) = 20.50, p < 0.001] were both significant. In the Goal section (illustrated in Fig. 2) the main effect of Reinforcement [F(1, 86) = 18.07, p < 0.001] and the interaction of Reinforcement × Days [F(9, 774) = 11.66, p < 0.001; quadratic component F(1, 86) = 40.55, p < 0.001] were also significant. The administration of CERON during acquisition led to an attenuation of the PREE, stemming both from decreased resistance to extinction of the PRF groups treated with CERON during acquisition, and from increased resistance to extinction of CRF animals treated with CERON during acquisition. The statistical analyses supported these conclusions as follows. There were significant quadratic components of the Drug in acquisition × Reinforcement × Days interaction in both the Run and Goal sections [F(1, 86) = 5.26 and 5.35, respectively, both p < 0.05]; the three-way interactions themselves also approached significance in both these sections [F(9, 774) = 1.74 and 1.71, p < 0.08 and p < 0.09, respectively]. The attenuation of the PREE appeared graphically to be clearest in the animals treated with CERON during both acquisition and extinction (see Fig. 2), but the four-way interaction was not significant and there were no other significant effects or interactions involving the factor of Drug in extinction.

Experiment 2

Rebaseline. The performance of the rats in the four treatment groups was closely matched during the rebaseline phase. The analyses revealed no significant effects of pre-exposure, nor of drug, either in latencies to first lick or in total number of licks.

Test. The performance at test of the rats in the four treatment groups was closely matched, up until the time that the tone was presented. The analyses revealed no significant effects of pre-exposure nor of drug either in latencies to first lick, or in time to complete licks 1-50, or licks 51-75. The times to complete licks 51-75 of the four groups were as follows: NPE-VEH, 9.39 s; PE-VEH, 5.74 s; NPE-CERON, 8.82 s; PE-CERON, 8.20 s.

The groups apparently differed in the times to complete licks 76-100 (see Fig. 3). There was clear LI in the vehicle condition: the pre-exposed animals exhibited less suppression of drinking during tone presentation than the non-pre-exposed controls. LI was abolished in the CERON condition: both the non-pre-exposed and pre-exposed animals showed equivalent suppression of drinking during the presentation of the tone. These outcomes were sup-
ported by a $2 \times 2$ ANOVA performed on the log times to complete licks 76-100 which yielded a significant main effect of Pre-exposure [$F(1,75) = 5.81, p < 0.02$] and an interaction of Pre-exposure $\times$ Drug which approached significance [$F(1,75) = 3.78, p = 0.056$]. Post-hoc $t$-tests based on the error term derived from the ANOVA revealed a significant difference between the pre-exposed and non-pre-exposed groups only in the vehicle condition [$t(75) = 3.12, p < 0.01$]. The same pattern appeared in the number of licks over 10 bins of 30 s during the presentation of the tone. As can be seen in Fig. 4 there was a clear LI effect in the vehicle-injected groups: the PE group drank more during the first 2 min of the tone than the NPE group. This difference was virtually non-existent in the CERON-injected groups. This was supported by $2 \times 2 \times 10$ ANOVA which yielded a significant interaction of Pre-exposure $\times$ Bins [$F(9,666) = 2.59, p < 0.01$], linear trend [$F(1,74) = 7.62, p < 0.01$], as well as by the significant linear trend of the Pre-exposure $\times$ Drug $\times$ Bins interaction [$F(1,74) = 4.10, p < 0.05$].

**DISCUSSION**

The administration of CERON in the acquisition stage of the PREE led to an attenuation of the PREE, so that in extinction, the drug-treated CRF animals had faster running speeds than vehicle-treated controls, while the drug-treated PRF animals had slower running speeds than their vehicle comparison group. This pattern appeared clearest when animals were given CERON in both acquisition and extinction. The administration of CERON in extinction alone exerted no influence on running speeds. This pattern of results does not at all resemble that found after the administration of haloperidol. First, in the one trial/day PREE procedure, haloperidol administered in acquisition either delays extinction in PRF animals or leaves it unaffected (Feldon et al., 1988; Rubin, 1991). Second, haloperidol speeds up extinction if administered only during that stage (Feldon and Weiner, 1988, 1991b; Rubin, 1991), which is a well-documented effect of neuroleptic drugs (Philips and Fibiger, 1979; Mason et al., 1980; Tombaugh et al., 1980). Thus, CERON did not resemble a neuroleptic drug in its effects on the PREE, whereas it has been shown to resemble a neuroleptic drug in its effects on LI (Weiner et al., 1992). Experiment 2, therefore, investigated the possibility that this inconsistency stems from the differences in the drug administration regimes used in the two paradigms, namely, chronic administration in the case of the PREE and acute administration in the case of LI. The results of this experiment showed that chronic administration of CERON not only failed to reproduce the drug-induced enhancement of LI seen with acute administration of the drug, but actually led to an abolition of LI. Thus, the behavioural effects of chronic CERON do not at all resemble those of acute administration.

To the best of our knowledge, nothing is known regarding differences in brain neurochemistry following acute vs chronic administration of CERON. As for suggesting possible mechanisms by which CERON might have produced the effects observed here, we can only offer a comparison with other treatments that have been shown to attenuate both PREE and LI, namely, anxiolytic drugs (Feldon et al., 1979; Feldon and Gray, 1981a), amphetamine (Weiner et al., 1985, 1987a), and lesions to the septo-
hippocampal system (Gray et al., 1978; Feldon and Gray, 1979a, b, 1981b; Rawlins et al., 1980, 1989; Feldon et al., 1985; Weiner et al., 1985, 1987a; Jarrard et al., 1986). The effects of CERON can be differentiated from those of anxiolytics in both the PREE and LI. Anxiolytics markedly slow down extinction when administered in extinction alone (Feldon and Gray, 1981a), and reduce suppression in both the PE and the NPE animals (Feldon and Weiner, 1989). Neither of these effects was evident with CERON. Amphetamine, like CERON, has been shown to attenuate the PREE when administered in acquisition, but it may differ from CERON because in the one trial per day procedure the attenuation produced by amphetamine resulted exclusively from decreased resistance to extinction in the PRF group even when amphetamine was given throughout acquisition and extinction (Weiner et al., 1985). However, it should be noted that in a multi-trial procedure, amphetamine when given throughout acquisition and extinction did increase resistance to extinction in CRF animals (Feldon et al., 1989). The amphetamine-induced attenuation of LI appears identical in form to that seen following chronic CERON administration in the present experiment (Weiner et al., 1981, 1984, 1988). Thus, the overall pattern of the data suggests a fairly close resemblance between the effects of chronic CERON and those of low doses of amphetamine on both the PREE and LI. In addition, the effects of chronic CERON on both these tasks resemble those of a variety of different lesions in the septo-hippocampal system (Ackil et al., 1969; Solomon and Moore, 1975; McFarland et al., 1978; Rawlins et al., 1980; Feldon et al., 1985; Jarrard et al., 1986; Kaye and Pearce, 1987a, b).

If resemblance in behavioural effects can be at all taken to reflect similar underlying neural substrates, then a likely candidate is increased dopamine (DA) transmission in the nucleus accumbens, which has been evoked to explain the LI-disruptive effects of both amphetamine and hippocampal lesions (Weiner, 1990; Gray et al., 1991). The effects of CERON on nucleus accumbens DA could be mediated via its action on CCK, since the latter is known to interact with DA in the nucleus accumbens (Weiss et al., 1988; Crawley, 1991). Alternatively, there might be some direct interaction between brain angiotensin II and DA mechanisms (Sudilovsky et al., 1983, 1989a, b; Braszco et al., 1988), although we found no interaction between 50 µg/kg CERON and 1.0 mg/kg d-amphetamine in earlier studies (Weiner et al., 1992). Clearly, neuropharmacological studies are needed to elucidate the brain mechanisms of the presently observed behavioural effects.

CERON has been reported to possess anxiolytic properties (Costall et al., 1990) as well as to improve cognitive functioning (Costall et al., 1989; Sudilovsky et al., 1989a). Neither the present results nor our earlier results with acute administration (Weiner et al., 1992) show any evidence of an anxiolytic action of this drug. As for cognitive enhancement, while facilitation of LI produced by acute administration of CERON could perhaps be interpreted as a reflection of cognitive enhancement, the present demonstration of a drug-induced attenuation of both the PREE and LI indicates that at least the chronic administration of this drug does not exert cognitive-enhancing effects.

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