Psychopharmacology (2002) 164:268–276
DOI 10.1007/s00213-002-1187-y

ORIGINAL INVESTIGATION

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Antalarmin, a putative CRH-RI antagonist, has transient reinforcing effects in rhesus monkeys

Received: 7 November 2001 / Accepted: 29 May 2002 / Published online: 5 September 2002

Abstract *Rationale:* During the course of our investigation of antalarmin, a corticotropin-releasing hormone (CRH) antagonist, in rhesus monkeys, we noticed that large, intravenous doses of antalarmin resulted in behavioral changes that resembled intoxication. *Objectives:* Antalarmin was evaluated in rhesus monkeys for its reinforcing effectiveness as well as for its effects on hypothalamic-pituitary-adrenal (HPA) axis activity. *Methods:* Twelve monkeys, each with a surgically implanted indwelling venous catheter, were trained to respond for and receive the short-acting barbiturate, methohexital. Monkeys responded on one of two schedules: a fixed ratio (FR) 10 (30 or 100), timeout (TO) 10 s schedule on which they received methohexital, antalarmin, vehicle or saline injections; or an FR30, TO 45 s during which saline, vehicle, or four different doses of methohexital or antalarmin were available. Each dose was available during a 25-min period separated by a 10-min TO. Blood samples were obtained from three monkeys before, during and after the self-administration sessions and assayed for ACTH and cortisol. *Results:* Antalarmin initially served as a reinforcer in 11 of 12 monkeys, although its reinforcing effects dissipated after three to four exposures under both operant schedules. Self-injection of antalarmin did not produce any change in cortisol levels, although methohexital did attenuate ACTH and cortisol release. *Conclusions:* This study provides the first evidence for transient reinforcing properties of a putative centrally acting CRH-R1 selective antagonist.

Keywords CRH antagonist · Antalarmin · *Macaca mulatta* · Schedule controlled responding · HPA axis · Cortisol · ACTH

Animals used in these studies were maintained in accordance with the "Principles of laboratory animal care" (NIH publication No. 83-23, revised 1985), and with the University of Michigan Committee on Animal Care and Guidelines of the Committee on the Care and Use of Laboratory Animal Resources, National Health Council (Department of Health, Education and Welfare, ISBN 0-309-05377-3, revised 1996)

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Introduction

Antalarmin, a close chemical analog of CP-154,526, is a non-peptidic corticotropin-releasing hormone (CRH) antagonist, selective for the CRH-R1 receptor subtype (antalarmin: Webster et al. 1996; CP-154,526: Schulz et al. 1996; Gottowik et al. 1997). CP-154,526 has been demonstrated to have central effects following systemic administration (Schulz et al. 1996), and antalarmin has been detected in cerebrospinal fluid following oral administration in rhesus monkeys (Habib et al. 2000). Antalarmin and CP-154,526 have been evaluated in rodent models of anxiety such as conditioned fear and elevated plus maze (antalarmin: Deak et al. 1999; CP-154,526: Chen et al. 1997; Griebel et al. 1998) and learned helplessness, a model for depression (antalarmin: Lundkvist et al. 1996; Schulz et al. 1996; Deak et al. 1999; CP-154,526: Mansbach et al. 1997).

Some similarities in the profiles of CRH antagonists and anxiolytics such as benzodiazepines have been noted in rat (Deak et al. 1999) and monkey studies (Habib et al. 2000). When diazepam and CP-154,526 were directly compared using models of anxious behavior in mice, the two compounds had similar anxiolytic activity (Griebel et al. 1998). In the case of the rhesus monkey, when only a transparent barrier separated male monkeys from one

another, the monkey that was pretreated with antalarmin showed a significant reduction in behaviors associated with anxiety and agitation (Habib et al. 2000). When we first began working with antalarmin in rhesus monkeys, we observed a profound behavioral change following intravenous injection of antalarmin. The monkeys lay down, or if they remained upright they supported themselves using the cage frame. They were virtually unresponsive to external stimuli such as sound, movement of an object into their field of vision, and direct physical contact. The lack of voluntary and reflexive movement was accompanied by pallor and excessive salivary secretion. These effects persisted for up to 30 min, with a gradual return of responsiveness that was accompanied by some ataxia and staggering. Recovery was uneventful and normal behavior resumed soon after. This appearance of intoxication led us to investigate antalarmin as a potential reinforcer using monkeys trained to self-administer the short-acting barbiturate, methohexital (e.g. Winger 1993).

The present study examines whether intravenous antalarmin supports drug-maintained responding when it is available on both single and multiple dose-delivery schedules in rhesus monkeys. The effects of self-administered antalarmin on ACTH and cortisol release were measured to examine whether intravenous antalarmin affects resting HPA axis activity.

Materials and methods

Subjects

A total of ten adult male rhesus monkeys (*Macaca mulatta*), weighing between 9.9 and 12.4 kg, and two intact female monkeys, weighing 7.8 and 8.6 kg, were the subjects for this study. All but two subjects (monkeys 3596 and 3580) had an extensive self-administration history with two or more classes of drug, including cocaine and methohexital. Monkeys 3596 and 3580, both males, had recently been trained to self-administer methohexital and had no prior drug self-administration history. The monkeys were fed 8–12 Purina Monkey Chow biscuits twice daily and fruit once daily to maintain normal adult weight. Water was freely available.

Apparatus

Each monkey was individually housed in a stainless steel cage measuring 83.3×76.2×91.4 cm deep (Bryan Research Equipment Corporation, Bryan, Tex., USA) located in a laboratory that contained a total of 24 similarly housed monkeys. The temperature in the room was maintained at 72°F, and lights were illuminated from 0630 until 1930 hours daily. Each monkey had an indwelling venous catheter in a femoral, internal, or external jugular vein. Catheters were inserted during aseptic surgery under ketamine (10 mg/kg) and xylazine (2 mg/kg) anesthesia. Following placement in the vein, the catheter was guided subcutaneously to the midscapular region, where it was externalized. The outer portion of the catheter was protected inside the cage by a flexible stainless steel tether, with one end attached to the double layer polyester jacket (Lomir, New York, N.Y., USA) worn by the monkey and the other bolted to the rear of the cage. Each cage had a 15×20 cm panel fixed to its right wall. Each panel had three stimulus lights, two red and one central green light, placed above two response levers. The red stimulus light over the right lever signaled drug availability. The green center light was illuminated for the duration of the drug injection, 1 ml every 5 s. During each timeout, all stimulus lights were extinguished and responding had no programmed consequences.

IBM/PS2 computers located in an adjacent room controlled the experiment. The computers were programmed using Med Associates software (Georgia, Vt., USA).

Procedure

Drug self-administration sessions were scheduled twice daily for 130 min starting at approximately 10 a.m. and 4 p.m., respectively. Saline was substituted on a frequent basis (25–50% of sessions). The reinforcing effectiveness of antalarmin (0.03, 0.1, 0.3 and 1.0 mg/kg per injection) was examined using the two operant schedules described below. All antalarmin substitutions were made during the morning session following one or two saline sessions the previous day.

Single-dose methohexital schedule

Five monkeys (three male) were tested using this schedule. Methohexital (0.1 mg/kg per injection), saline or antalarmin (0.03, 0.1, 0.3 or 1.0 mg/kg per injection) delivery was contingent on the monkey emitting the required number of lever presses [fixed ratio (FR)=10 (n=3), 30 (monkey 2487) or 100 (monkey 3147)], and there was a 10-s timeout (TO) between each injection and the next response opportunity. The FR values differed among subjects in order to obtain a reliable difference between the number of methohexital and saline injections administered by each monkey (saline responding <15% methohexital responding). A stable baseline of self-administration behavior was defined as consistency (<10% variability) across sessions in responding for methohexital or saline. The first dose of antalarmin tested was 0.1 mg/kg per injection. Thereafter, the order of presentation of the antalarmin doses was varied randomly. Antalarmin was tested no more than once every 3 days for one monkey (3574), and at 7-day intervals or greater for the remaining four monkeys. Each antalarmin dose was substituted on one to three occasions for each subject. Blood samples were obtained from four monkeys during saline, antalarmin-vehicle and antalarmin tests. The sampling times were 15 and 5 min prior to the start of the session, and then during the session at 25, 60, 95 and 130 min. Blood was also sampled at 35, 70, 130 and 190 min after the session had ended.

Four-dose methohexital schedule

Seven male monkeys were tested using this schedule. Four doses of methohexital (0.01, 0.03, 0.1 and 0.3 mg/kg per injection) were available on an FR30 TO 45 s schedule of reinforcement (e.g. Winger et al. 1989). Each session was divided into four components, with each component lasting either 25 min or the time taken to self-administer 20 injections, whichever occurred first. Each component was separated by a 10-min TO. The doses of methohexital were presented in ascending order. The dose of drug was controlled by injection duration. Methohexital was diluted to 0.1 mg/kg and the doses (and infusion duration) were 0.01 (0.5 s), 0.03 (1.7 s), 0.1 (5 s) and 0.3 mg/kg (16.7 s). During sessions when saline was available for self-administration, saline was available during all four components and the injection duration varied in each component to mimic the duration of the drug injections. When testing criteria were met (the response-rates for methohexital showed a reliable, inverted U-shaped function and saline responding did not exceed 0.5 response/s on any component), antalarmin (0.03, 0.1, 0.3 and 1.0 mg/kg per injection) or vehicle was substituted and doses were tested in ascending order. Antalarmin and its vehicle were substituted on five occasions, at intervals of 4–8 days. Methohexital and saline were also tested on five occasions for comparison with antalarmin. Blood samples were

obtained from three monkeys during saline, methohexital, antalarmin-vehicle and antalarmin tests. The sampling times were 15 and 5 min prior to the start of the session, and then during the session at 25, 60, 95 and 130 min. Blood was also sampled at 35, 70, 130 and 190 min after the session had ended.

Blood collection and handling

Blood samples were collected from a total of seven monkeys in the self-administration study via their venous indwelling catheters. Prior to drawing each blood sample, a 3 cc syringe was used to empty the contents of the catheter and this blood-containing fluid was discarded. Then each blood sample (1.1–1.4 ml) was placed in a 2 ml Vacutainer (Becton Dickinson and Company, Franklin Lakes, N.J., USA) containing 0.04 ml of 7.5% EDTA and immediately placed on ice. After a blood sample was drawn, 1.5–3 ml of 30 IU/ml heparin saline solution was injected into the catheter and, for samples taken during the session, the lumen of the catheter was re-filled with drug or vehicle.

Blood samples were centrifuged at 4000 r.p.m. for 5 min at 4°C and then the plasma (0.7 ml) was pipetted into 2 ml Cryovials (Corning Incorporated, Corning, N.Y., USA) and stored at –80°C until assay. Cortisol and ACTH levels were determined using commercially available radioimmunoassay kits (cortisol: Diagnostic Products Corporation, Los Angeles, Calif., USA; ACTH: Nichols Institute Diagnostics, La Jolla, Calif., USA).

Data analysis

Single-dose methohexital schedule

Antalarmin self-administration data (number of injections) are presented in raw form. The average number of and response rates for methohexital and saline injections for each subject are also presented for comparison. The results of the saline and 0.1 mg/kg per injection methohexital tests that occurred during sessions immediately prior to each antalarmin substitution are used for comparison with antalarmin in the data analysis. Mean and individual data are presented for each dose of antalarmin. The data are analyzed for differences in the numbers of injections taken during the initial versus later presentations of antalarmin.

Mean cortisol and ACTH data for four monkeys, representing the time course of changes in cortisol and ACTH release during saline, antalarmin-vehicle and antalarmin self-administration on the four-dose methohexital schedule, are also presented.

Four-dose methohexital schedule

The rates and injection numbers for these monkeys are presented as raw data, with the data for each drug condition either being averaged across all five tests, or test result presented separately. The total drug intake for the methohexital and antalarmin tests was calculated by multiplying the number of injections taken during each component by the dose available during that component, and then adding the intake for each of the four components.

Mean cortisol and ACTH data for three monkeys, representing the time course of changes in cortisol and ACTH release during saline, methohexital, antalarmin-vehicle and antalarmin self-administration on the four-dose methohexital schedule, are also presented.

Drugs

Antalarmin was synthesized as described by Webster et al. (1996) and dissolved in a vehicle containing 9% ethanol, 9% emulphor and 82% sterile water at a concentration of 20 mg/ml immediately prior to use. The antalarmin solution was further diluted with sterile saline to provide between 0.03 and 1.0 mg/kg per 1 ml injection. Methohexital was purchased from Ace Surgical Supplies (Brockton, Mass., USA) and diluted with sterile water.

Statistics

All data are presented as either individual data or as mean±SEM. Analysis of variance (ANOVA) was conducted on raw (rate, injection and intake) or standardized data (cortisol and ACTH – where the pre-session values were averaged and then subtracted from the values of samples obtained during and after the session). Drug, session component and test number were within-subject variables. Where appropriate, post-hoc pairwise comparisons, using the Tukey Honest Significant Difference test of significance, were used ($P<0.05$). All statistical analyses were carried out using Statistica (v.5.0, Statsoft, Tulsa, Okla., USA).

Results

Single-dose methohexital schedule

Antalarmin served as a reinforcer in four of five monkeys the first time that two doses (0.1 and 0.3 mg/kg per injection) were made available for self-administration (Fig. 1, Table 1). The exception was monkey 3596, whose responding for antalarmin could not be differentiated from saline-maintained responding. Responding for antalarmin generated an inverted U-shaped function, and the response rates generated when 0.03 and 1.0 mg/kg per injection antalarmin were available for self-administration were not different from saline-maintained responding (Fig. 1). When 1.0 mg/kg per injection antalarmin was available, several of the animals were visibly intoxicated during and after the session. In tests where monkeys were first exposed to the four antalarmin doses, overall responding for 0.1 and 0.3 mg/kg per injection antalarmin clearly exceeded saline-maintained responding. During subsequent tests with these doses (after three or more exposures to various doses of antalarmin), the reinforcing effects of doses that had previously been self-adminis-

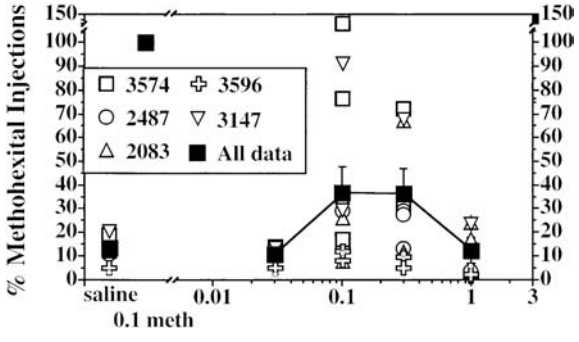

Fig. 1 Number of injections of antalarmin and saline earned during the self-administration sessions, expressed as a percentage of the number of injections of the training drug, 0.1 mg/kg per injection methohexital, that were earned during the previous session. Mean data are shown by the *filled squares* (n=5) and individual data with *open symbols*. Monkeys worked on an FR10 (n=3), FR30 or FR100 (n=1 for each) TO 10-s schedule twice daily, commencing at 10 a.m. and 4 p.m.

Table 1 Responding for antalarmin during early versus later exposures to 0.1 and 0.3 mg/kg per injection doses on the single-dose methohexital schedule

Monkey	Sex (M/F)	Methohexital injections (mean±SEM)	Response rate (per s)	Saline injections (mean±SEM)	Response rate (per s)	0.1: injections		Intake (mg/kg)		0.3: injections		Intake (mg/kg)	
						Early	Later	Early	Later	Early	Later	Early	Later
2487[a]	F	112.3±8.03	0.56±0.05	12.1±2.09	0.05±0.01	39	12	3.9	1.2	33	15	9.9	4.5
3574	M	99.3±5.91	0.15±0.01	18.9±2.21	0.03±0.00	76	15	7.6	1.5	72	32	21.6	9.6
3147[a]	M	60.4±2.50	0.88±0.04	12.2±2.40	0.16±0.03	55	19	5.5	1.9	41	8	12.3	2.4
2083	F	104.0±6.21	0.17±0.01	12.4±1.49	0.02±0.01	27	8	2.7	0.8	70	12	21	3.6
3596	M	132.5±5.21	0.24±0.02<?2>	7.0±1.45	0.01±0.00	16	16	1.6	1.6	13	7	3.9	2.1
					Mean (n=5)	42.6*	14.0	4.26	1.40	45.8*	14.8	13.74	4.44
					SEM (n=5)	10.6	1.9	1.06	0.19	11.3	4.5	3.38	1.36

[a]Fixed ratio was ten lever presses except for monkeys 2487 (FR30) and 3147 (FR100)

*Injections and intake on early exposure to antalarmin exceeded number earned on later exposure, $P<0.05$

Fig. 2 Cumulative injections of antalarmin or vehicle earned over the 2 h 10 min self-administration session. Initial exposure to antalarmin resulted in delivery of a larger number of injections that were distributed across the session (*open squares*, n=4; *$P<0.05$). When given the opportunity to self-administer antalarmin after four or more exposures to the drug (*circles*), the pattern of injections resembled responding for vehicle (*closed squares*) for both 0.1 (*upper panel*) and 0.3 mg/kg per injection antalarmin (*lower panel*). Monkeys responded for drug or vehicle on an FR10 (n=2), FR30 (n=1) or FR100 (n=1) TO 10-s schedule

tered were observed to diminish in each of the four monkeys whose behavior was originally maintained by antalarmin. The data for the 0.1 and 0.3 mg/kg per injection antalarmin doses for each monkey were divided on the basis of whether they were generated during early versus later exposures to antalarmin. These data (number of injections and drug intake) are shown in Table 1. Early versus later responding for antalarmin was significantly different for both the 0.1 and 0.3 mg/kg per injection doses [0.1 mg/kg per injection: $F(1,4)=9.02$, $P<0.05$; 0.3 mg/kg per injection: $F(1,4)=7.81$, $P<0.05$]. This apparent loss of reinforcing effect was not overcome when the dose of antalarmin was increased to 1.0 mg/kg per injection.

Cumulative injections that were earned during the self-administration sessions are shown in Fig. 2. When antalarmin was first made available to the four monkeys

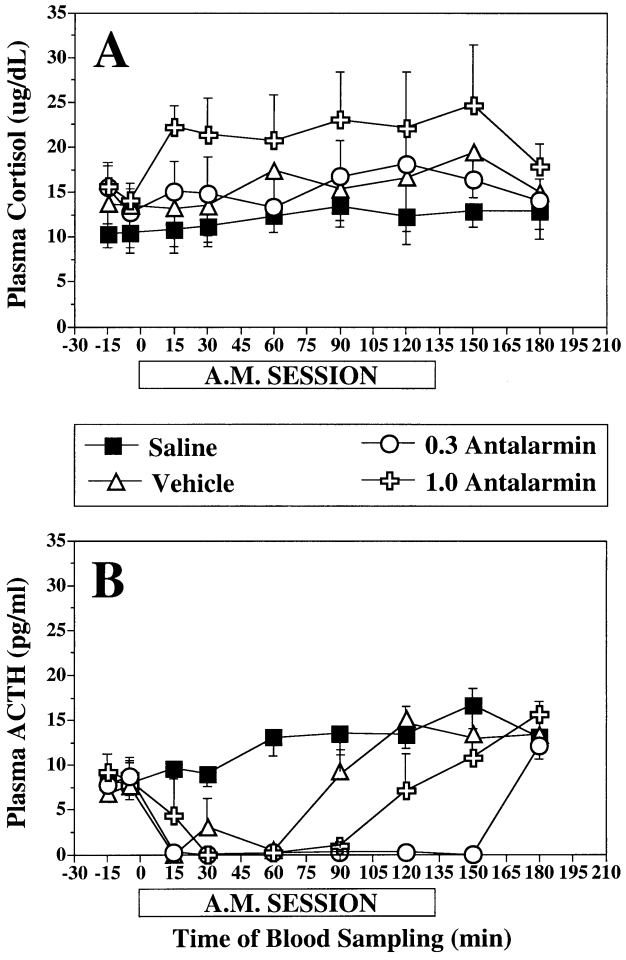

Fig. 3 Plasma cortisol (**A**) and ACTH (**B**) levels following self-administration of saline, antalarmin-vehicle or antalarmin on a single-dose schedule of reinforcement. **A** Cortisol levels were unchanged during self-administration of saline. **B** ACTH levels decreased during self-administration of antalarmin or vehicle relative to when saline was available ($P<0.05$). The lack of effect of low ACTH levels on cortisol secretion in the presence of antalarmin and its vehicle is indicative of a problem with the measurement of ACTH rather than of a reduction in its secretion from the pituitary gland. For methodological details, see Fig. 1

in which it initially served as a reinforcer, the pattern of injections of 0.1 mg/kg per injection (Fig. 2A) and 0.3 mg/kg per injection antalarmin (Fig. 2B) differed both in number [$F(5,10)=5.11$, $P<0.05$] and distribution of injections [$F(5,10)=12.40$, $P<0.05$] relative to those earned during vehicle or in later antalarmin tests. During the initial exposures to antalarmin, monkeys earned more injections at both doses and the injections were distributed across the session rather than concentrated within the first 15 min.

Blood samples were obtained before, during and after methohexital, saline, antalarmin-vehicle and antalarmin self-administration sessions in four of the five monkeys that were tested using the single-dose schedule of reinforcement. The cortisol and ACTH data are summarized in Fig. 3A, B. The mean pre-session cortisol and ACTH ranges were 10–15 μg/dl and 6–9 pg/ml, respectively. Saline, antalarmin-vehicle and antalarmin had no effect on plasma cortisol levels (Fig. 3A). It is evident, however, that the presence of both antalarmin and its vehicle interfered with the measurement of ACTH in blood samples taken during the self-administration session. During the time that antalarmin or its vehicle was being self-administered, ACTH levels were low or undetectable. There is no evidence that this was due to an effect of antalarmin on ACTH secretion, as vehicle produced the same effect and more particularly, cortisol levels remained unaffected during the apparent absence of ACTH. The ability to measure ACTH in plasma was restored at the cessation of responding for antalarmin or its vehicle.

Four-dose methohexital schedule

Methohexital was an effective reinforcer under the schedule conditions used in this study. Responding for methohexital was dose dependent, with rates peaking at 1.26±0.11 responses per second for 0.1 mg/kg per injection methohexital (Fig. 4A), resulting in 18.63±0.39 injections (maximum possible=20; data not shown). By contrast, responding for saline or vehicle was mostly flat across the four components of the self administration session, averaging no more than 4.84±0.73 (saline) and 8.33±1.20 (vehicle) injections per component. There was a significant effect of drug on both rates of responding [$F(3,12)=21.36$, $P<0.05$] and injection delivery [$F(3,12)=25.92$, $P<0.05$], as rates for and injections of methohexital exceeded those for saline and vehicle (in component 2) as well as antalarmin (in component 3, $P<0.05$). When the responding for antalarmin was averaged across trials, the dose-response function fell between those of methohexital and saline/vehicle and there was no dose at which responding clearly peaked (Fig. 4A). However, when antalarmin-maintained responding across the five tests was examined, there was a significant difference between the tests [$F(4,16)=4.34$, $P<0.05$], with animals responding for antalarmin at a somewhat higher rate during the first test than during the third and fifth tests ($P<0.05$; Fig. 4B). Similarly, the number of antalarmin injections that was earned during the first test exceeded those earned during test 5 ($P<0.05$; data not shown). When the data were examined across tests, responding for antalarmin was somewhat dose-dependent, with the number of injections earned during the third component exceeding those earned during the fourth ($P<0.05$; data not shown). There were no differences in responding for methohexital or saline across the five tests (data not shown). The response rates for antalarmin-vehicle during the first component varied with repeated testing [$F(3,12)=8.92$, $P<0.05$], as responding for vehicle during the first test exceeded vehicle-maintained responding during tests 3, 4 and 5 ($P<0.05$; data not shown).

Total intake (mg/kg) of self-administered methohexital and antalarmin on the five occasions during which each

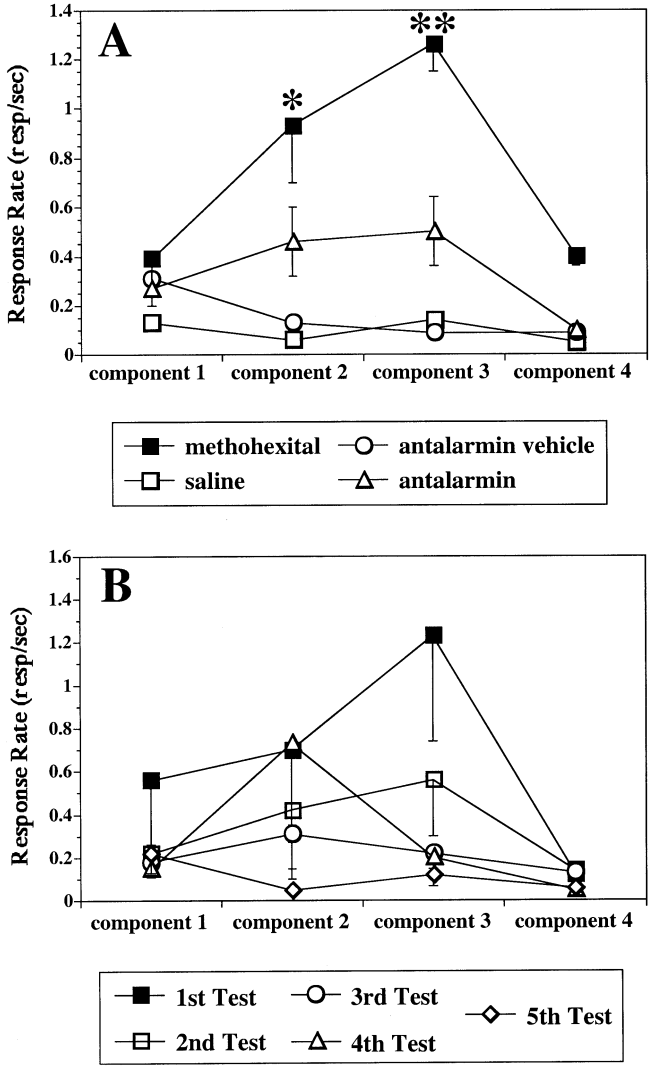

Fig. 4A, B Data for seven monkeys trained to a four-component schedule during which 0.01, 0.03, 0.1 or 0.3 mg/kg per injection methohexital or saline was available on an FR30 TO 45 s schedule. Each component lasted either 25 min or 20 injections (whichever elapsed first), with a 10-min TO between components. Antalarmin (0.03, 0.1, 0.03 and 1.0 mg/kg per injection) or vehicle was substituted for methohexital on five occasions, and doses were tested in ascending order. **A** Comparison of response rates for methohexital, saline, vehicle and antalarmin averaged across tests. * Methohexital versus saline and vehicle (P<0.05); ** Methohexital versus saline, vehicle and antalarmin (P<0.05). **B** Rates of responding for intravenous injections of antalarmin (0.03, 0.1, 0.3 and 1.0 mg/kg per injection) on five successive occasions. Each dose was available during one component of the session, and doses were tested in ascending order. The response rates differed across tests, with lower rates being measured during tests 3 and 5 relative to rates generated during test 1 (P<0.05)

drug was tested is presented in Fig. 5. Methohexital intake did not vary across the five tests, averaging between 16 and 18.5 mg/kg. Antalarmin intake peaked on the first occasion that it was made available (10.9±1.6 mg/kg) and steadily declined with successive testing. The total intake of antalarmin during tests 4 and 5 was significantly lower than during test 1 (P<0.05).

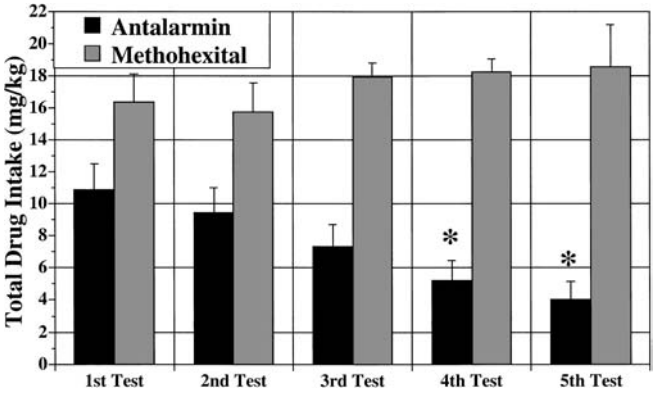

Fig. 5 Comparison of total drug intake across successive trials in which methohexital or antalarmin were available for self-administration (n=7). Methohexital intake did not differ across tests, but antalarmin intake decreased with each test. *Test 1 versus tests 4 and 5 (P<0.05). For additional details, see Fig. 2

Blood samples were obtained before, during and after methohexital, saline, antalarmin-vehicle and antalarmin self-administration sessions in three of the seven monkeys that were tested using the four-dose schedule of reinforcement. The cortisol and ACTH data are summarized in Fig. 6A and B. The mean pre-session cortisol and ACTH levels were 12.60±1.10 μg/dl and 6.08±0.90 pg/ml, respectively. Antalarmin injections had no effect on resting cortisol levels relative to methohexital, saline or antalarmin-vehicle, regardless of total drug intake. Although there were no overall differences among the treatments on cortisol secretion, a significant interaction between treatment and sampling time [F(21,42)=2.95, P<0.005] revealed that methohexital self administration reduced cortisol release relative to when saline and vehicle were available for self administration (P<0.05). The presence of both antalarmin and its vehicle interfered with the measurement of ACTH in blood samples taken during the self-administration session, as the assay was unable to detect ACTH in samples collected during this time (data not shown). This did not appear to be a direct effect of antalarmin or vehicle on pituitary function, as cortisol levels remained elevated during the hours that ACTH was unable to be measured (see also Fig. 3B). As with cortisol, methohexital self-administration attenuated ACTH release relative to when saline was available for self-administration (P<0.05).

Discussion

The CRH-R1-selective antagonist, antalarmin, initially served as a reinforcer in the majority of monkeys trained to a methohexital baseline. Four out of five monkeys responded for antalarmin at two doses (0.1 and 0.3 mg/kg per injection) at rates exceeding those for saline, on a single-dose methohexital schedule. All seven monkeys trained on a four-dose methohexital schedule responded for antalarmin at rates exceeding saline or vehicle-

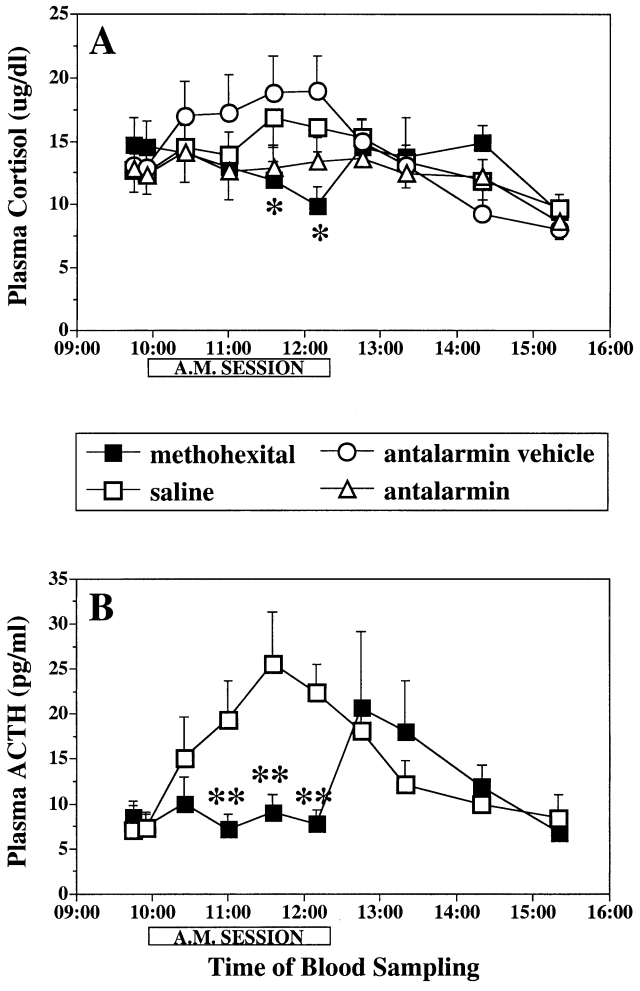

Fig. 6 Plasma cortisol (**A**) and ACTH (**B**) levels following self-administration of methohexital, saline, antalarmin-vehicle and antalarmin on a four-dose schedule of reinforcement. **A** Cortisol levels decreased during self-administration of methohexital relative to when saline (*) or vehicle (**) was available ($P<0.05$). **B** ACTH levels decreased relative to when saline was available for self-administration (*$P<0.05$). ACTH was unable to be measured in the presence of antalarmin or its vehicle. However, it is likely that ACTH was still being secreted as cortisol levels (**A**) did not diminish during the session. For methodological details, see Fig. 3

maintained behavior. The responding across the doses of antalarmin appeared to be somewhat dose-dependently distributed, peaking at 0.1 or 0.3 mg/kg per injection. This finding is not without precedent. In an earlier study, we examined the reinforcing efficacy of a number of glucocorticoid agonists using a similar procedure. In this study, dexamethasone served initially as a reinforcer in all subjects, but this effect dissipated in all but one subject on repeated testing (Broadbear et al. 1999). In the present study, the lack of drug self-administration history may have been a factor in the outcome for the one monkey that did not respond for antalarmin (3596). However, of the monkeys that did self-administer antalarmin, one monkey (3580) similarly lacked extensive experience with the drug self-administration procedure.

The reinforcing efficacy of antalarmin diminished unexpectedly after three or four exposures, resulting in self-administration behavior that was not significantly different from responding generated for vehicle or saline. This observation might be explained by the development of tolerance or sensitization to the effects of antalarmin following repeated exposure. However, this conclusion was not supported by our observations. Antalarmin-maintained responding was not restored by increasing or lowering the dose that was available for self-administration, as would be predicted if the loss of reinforcing effect was due to a change in sensitivity to the effects of antalarmin. The sedative effects that followed a single intravenous injection of 10 mg/kg antalarmin (unpublished observations mentioned in the Introduction) were also transient in nature, as they too diminished following four or five exposures to antalarmin. The sedation was accompanied by an increase in the secretion of ACTH and cortisol, which also subsided with repeated testing (Broadbear et al., unpublished data). The physiological determinants of these transient effects are a matter for speculation. Antalarmin has been shown to enter the CNS following oral administration to rhesus monkeys (Habib et al. 2000); therefore it is likely that the sedative and rewarding effects that we have measured following administration of antalarmin may be mediated via extrahypothalamal CRH receptors in the CNS. Some uncertainty remains though, as the present study does not show a clear inhibitory effect of antalarmin at pituitary CRH-R1 receptors (as inferred from antalarmin's lack of effect on resting cortisol levels). However, this in no way excludes the involvement of a central CRH-R1 mechanism from consideration.

There are no previous reports in which CRH antagonists have been tested as reinforcers in paradigms that measure reinforcing effectiveness. The comparatively recent advent of non-peptidic CRH antagonists that permeate the blood-brain barrier following peripheral administration, as well as the lack of a theoretical framework for such a prediction, make this observation a novel and surprising one. A clue as to the utility of CRH antagonists as reinforcers may lie in a study by Cador and co-workers (Cador et al. 1992) in which CRH was administered intracerebroventricularly (ICV) or subcutaneously (SC) in rats trained in a conditioned place preference paradigm. Rats spent less time in the environment that had been paired with CRH presentation, and this effect was independent of CRH's stimulation of ACTH and corticosterone release. CRH produced place aversion more potently following administration via the ICV route. This effect was blocked by prior ICV administration of the CRH antagonist, α-helical CRH_{9-41}, implying that the aversive effect of CRH may have been mediated by a population of CRH receptors independent of those mediating its neuroendocrine effect. The study, however, did not report whether α-helical CRH_{9-41} itself affected conditioned place preference. More recent studies have examined whether CRH antagonists affect cocaine self-administration (Goeders and

Guerin 2000) and reinstatement of heroin- and cocaine-maintained responding (Shaham et al. 1998) in the rat. When rats were acutely pretreated with CP-154,526, a close chemical analog of antalarmin, a decrease in ongoing cocaine self-administration was observed in the absence of an effect on food-maintained behavior. Curiously, pretreatment with CP-154,526 reduced responding to a greater degree than was seen when saline was substituted for cocaine (Goeders and Guerin 2000). This finding, together with the finding by Shaham and coworkers (1998) that acute CP-154,526 pretreatment attenuated the foot-shock induced reinstatement of both cocaine and heroin responding, could be interpreted as being attributable to behavioral effects of the CRH antagonist itself. For instance, CRH antagonists such as CP-154,526 or antalarmin have been likened to benzodiazepines (Lundkvist et al. 1996; Griebel et al. 1998) in measures of anxiolytic activity. Griebel and coworkers (1998) compared the behavioral effects of CP-154,526 with those of buspirone, a 5-HT_{1A} partial agonist, and diazepam, in a variety of procedures designed to measure anxiolytic activity. CP-154,526 had anxiolytic effects that were similar to diazepam in measures of anxiety in mice, although CP-154,526 had a narrower spectrum of anxiolytic activity. In addition, CP-154,526 and the benzodiazepine, chlordiazepoxide, both attenuated cocaine-maintained behavior to levels resembling saline-maintained responding (Goeders et al. 1989; Goeders and Guerin 2000). When considering the apparent reinforcing effects of antalarmin following acute substitution for methohexital in the monkey (present study), it may be that a benzodiazepine-like drug-state associated with centrally acting CRH antagonists is contributing to antalarmin's acute effects. Based on the results of the present study, it would be interesting to revisit studies in which CRH antagonists have been used acutely in models of anxiety or reinstatement of drug-seeking in mice and rats to determine whether their effectiveness diminishes with chronic treatment. The reinforcing effectiveness of antalarmin that dissipated with repeated exposure in the present study may be linked to these other behavioral effects.

Acute intravenous administration of antalarmin did not attenuate basal plasma cortisol levels, despite reports of its having high affinity and selectivity for CRH-R1 receptors (Webster et al. 1996; Gottowik et al. 1997). Blockade of CRH-R1 receptors typically attenuates increases in ACTH and cortisol following exogenous administration of CRH (Schulz et al. 1996; Webster et al. 1996; Broadbear et al., unpublished data) or cocaine (Broadbear et al. 1999a). However, antalarmin's lack of effect on cortisol secretion in the present study concurs with earlier reports that chronic administration of antalarmin was necessary before reductions in basal corticosterone levels could be detected in rats (Bornstein et al. 1998; Wong et al. 1999). Although the HPA axes of the animals in the current study were not stimulated experimentally, the preparation that we used was sensitive enough to detect a decrease in ACTH and cortisol release. Methohexital self-administration resulted in decreases in both ACTH and cortisol relative to when saline was available for self-administration. During the measurement of plasma ACTH during the self-administration session, we were unable to measure ACTH in blood samples obtained while antalarmin or its vehicle being actively self-administered. The apparent reduction in ACTH levels is most likely due to a problem with the experimental procedure rather than to a reduction in pituitary secretion. Several observations point to this conclusion. Despite the apparent drop in ACTH, cortisol levels remained unchanged during the session. In addition, the very low ACTH levels were restored to pre-session levels immediately after the antalarmin or vehicle session ceased to be self-administered.

In summary, this series of studies demonstrates that the CRH-R1 selective antagonist, antalarmin, temporarily maintained self-administration behavior in 11 out of 12 rhesus monkeys when substituted for methohexital using two different operant schedules. After three or four exposures to antalarmin, its reinforcing effect appeared to wane, and increasing or lowering the dose of antalarmin that was available for self-administration did not reverse this effect. Basal cortisol release was unaffected during self-injection of antalarmin, whereas both ACTH and cortisol levels were attenuated by self-administration of methohexital, the training drug used in this study. We have observed that intravenous antalarmin produces a range of behavioral changes, including sedation, ataxia and excessive salivation. Whether this profile of effects is specific to antalarmin, or is due to the blockade of central CRH-R1 receptors and therefore able to be reproduced using other centrally acting CRH-R1 selective antagonists, is yet to be determined.

Acknowledgements The authors wish to thank Nicholas Jones, Karen Wiesenauer, Deborah Huntzinger, Sarah Pilkington, Jolan Terner and Laurie McDowell for their expert technical assistance. The authors also extend their thanks to Drs. Jack Mendelson and Nancy Mello for their helpful discussions regarding these data. The United States Public Health Service Grant DA 09161 supported this work.

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