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# Effects of a Non-peptide CRF Antagonist (DMP696) on the Behavioral and Endocrine Sequelae of Maternal Separation

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*We examined whether blockade of corticotropin-releasing factor (CRF) receptors by a non-peptide CRF antagonist (DMP696) would attenuate the stress hyper-responsiveness that occurs in response to maternal separation. In a social interaction test as well as the elevated plus maze, adult male rats, which had been maternally separated as infants, displayed more anxiety-like behavior compared with handled rats. DMP696 increased social interaction in both groups. In the elevated plus maze however, DMP696 significantly increased open arm time in the maternally separated rats but not in the handled group whereas chlordiazepoxide increased open arm time in both groups. DMP696 also appeared to block stress-induced ACTH secretion more readily in the maternally separated group compared with the handled rats. These observations suggest that CRF antagonists are particularly effective in animals that are hyper-responsive to stress and may therefore have utility in the treatment of anxiety and affective disorders where CRF has been implicated.*

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KEY WORDS: *Corticotropin releasing factor; Maternal separation; Stress; Antagonist; Plus maze; ACTH; Rat*

Disruption of the maternal infant relationship can result in long-lasting changes in stress responsiveness. For example, repeated long periods of maternal separation (MS) of rodents during early development can produce increased ACTH and corticosterone levels in response to mild stressors as an adult (Plotsky and Meaney 1993). In addition, rats that experienced prolonged maternal separation display increased anxiety-like behavior in various behavioral models including defensive withdrawal, elevated plus maze and open field (Ladd et al. 2000). In contrast, rat pups subjected to brief separations (early handling) show reduced endocrine responses to stress (Levine 1957; Meaney et al. 1989) and also appear to be less fearful and anxious behaviorally (Meerlo et al. 1999). Whereas the behavioral effects of early handling have been robust and reproducible, the behavioral consequences of maternal separation appear to be more variable and dependent upon many as yet unspecified variables (Lehmann and Feldon 2000).

The increased stress responsiveness following maternal separation may depend, at least in part, on increased levels of corticotropin releasing factor (CRF) in the brain. Adult rats which experienced maternal separation for 3 h/day during postnatal days 2–21 had increased levels of CRF mRNA in the hypothalamic paraventricular nucleus (PVN) and central nucleus of the amygdala (Plotsky and Meaney 1993). Similar increases

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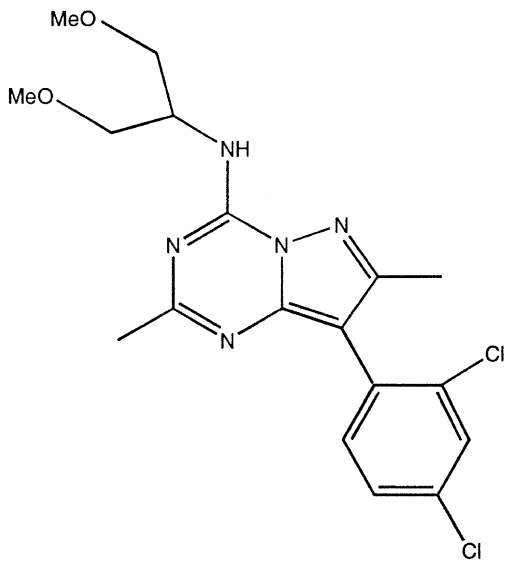
in basal levels of CRF mRNA in the PVN, the endocrine responses to restraint, and anxiogenic behavior occur in pups that had been separated for 8 h every other day between postnatal day (PND) 2 and PND 10 (Patchev et al. 1997). While undoubtedly maternal separation causes a number of neurochemical changes, the increased CRF levels are particularly intriguing given its central role in controlling stress responsiveness. CRF has been shown to regulate many behavioral aspects of the stress response (Koob and Heinrichs 1999). For example, i.c.v. administration of CRF induces anxiety-like behavior as measured in such paradigms as the elevated plus maze, shock-induced freezing, defensive withdrawal and social interaction. Likewise, i.c.v. administration of peptidergic CRF antagonists block the behavioral effects induced by stress or the infusion of CRF. The consistent preclinical results as well as clinical data indicating that CSF CRF levels are elevated in melancholic depression (Nemeroff et al. 1984) have led to speculation that a non-peptide CRF antagonist might prove to have antidepressant and anxiolytic properties (Holsboer 1999; Gilligan et al. 2000). Encouraging results from the first open label clinical trial (Zobel et al. 2000) support the preclinical predictions (Mansbach et al. 1997) that non-peptide CRF antagonists may indeed prove useful in treating depression and anxiety.

Here we addressed the question whether a CRF antagonist will demonstrate anxiolytic efficacy in rats whose behavior has been modified by neonatal adverse experiences. We utilized a novel, non-peptidergic CRF antagonist, DMP696 (Figure 1) which is a high affinity pure antagonist at the $CRF_1$ receptor (Ki = 1.6 nM) with little or no binding affinity for the $CRF_2$ receptor, CRF-binding protein or any of 70 other receptors examined (He et al. 2000). DMP696 has previously been shown to suppress ACTH secretion and to possess potent anxiolytic effects in the defensive withdrawal paradigm (He et al. 2000). Similar non-peptidergic, orally bioavailable, CRF antagonists have been shown to have antidepressant-like properties (Mansbach et al. 1997) and to be anxiolytic in the elevated plus maze (Griebel et al. 1998). However, most if not all of these studies have been conducted in normal laboratory rats. Here we examined the hypothesis that administration of a CRF antagonist would reverse some of the endocrine and behavioral consequences of the (Patchev et al. 1997) maternal separation paradigm in which infant rats are separated from their mother for 8 h every other day from day 2 to day 10. The results indicate that compared with an early handled control group, DMP696 can reduce anxiety-like behavior resulting from maternal separation.




DMP696

**Figure 1.** Structure of the CRF antagonist DMP696.

## METHODS

### Maternal Separation

Long-Evans rats from Charles River (Chicago, Illinois) were bred in our facility. Pregnant females were individually housed in transparent polycarbonate cages and exposed to a 12 h light/dark cycle with free access to food and water. One day after parturition (postnatal day 1 or PND1), each litter was culled to five male and five female pups, and placed in clean cages. The litters were then allocated to either the 10-min handled or 8-h separated group. The 8-hour separation or 10-min handling procedure started on PND 2 and continued every other day until PND 10 (total of five separation sessions). At the time of separation, the dam was removed from the home cage to another box, where she remained for each separation episode. Pups were removed from nest cages and placed in a new cage with littermates. During separation, pups were placed on heating pads and maintained at 30-33°C in a novel room, separate from where the dam was kept. Neither food nor water was available during the deprivation period. At PND 21 male pups were weaned and housed in groups of two or three with their littermates. The pups were handled once every 2 weeks during cage changing. All experiments met NIH guidelines and were approved by DuPont Pharmaceutical animal care and use committees.

### Social Interaction

Only male rats (PND 60–65) were tested in the social interaction test. The test was conducted in a stainless steel arena with a height of 30.4 cm and a diameter of 118 cm. The illumination on the floor of the tank was 4 scotopic

lux. Two days prior to testing, the rats were placed individually in the test arena for 10 min of conditioning each day. Testing consisted of pairing unfamiliar male rats of the same separation group but from different litters for 10 min in the test arena under high light conditions of 1080 scotopic lux. A camera was mounted vertically above the arena and the rats were observed on a video monitor in an adjacent room. Interaction between the pair was timed by viewing the following behaviors simultaneously: sniffing, grooming each other, following, jumping on, wrestling and crawling under or over. The total time spent engaged in these activities over a 10 min period was used as a measure of social interaction. Ten pairs of rats were used in each group. Rats were not fasted, nor were they acclimatized to the room.

### Elevated Plus Maze

Another group of male rats (PND 60–65) was tested in the elevated plus-maze which consisted of four arms radiating from the center platform. The arms were 10 cm wide and 60 cm long. Two of the arms were open (no sides), and two were enclosed (made of black plexiglass). The center platform was semi-open. The whole maze was on a stand 50 cm above the floor. Rats were individually placed in the center platform and the behavior was monitored over a period of 5 min using a video camera and monitor. The illumination in the open arms, closed arms and center was 100, 8, and 25 scotopic lux respectively. We measured the total time spent and number of entries into the open and closed arms over the 5-min period. Time in the center platform was not counted as time spent in either open or closed arms and therefore the total time (open plus closed) did not always add up to 5 min. Entry into an open or closed arm began when the rat put all four paws into that arm while exit from the arm occurred when at least two paws came out. The percent ratios between the number of entries or time spent in open arms versus total number of entries or time spent in both types of arms were computed for each individual rat and used as a measure of anxiety. Each group consisted of 11–13 non-fasted male rats that had not been acclimatized to the room or plus maze apparatus.

### Endocrine Responses to Stress

About one week after testing in the elevated plus maze, the rats were subjected to a single mild footshock (1.5 mA for 1 s) which was delivered through a wire grid on the cage floor. Five minutes later the rats were sacrificed by decapitation, and trunk blood collected in tubes containing EDTA for measurement of ACTH by radioimmunoassay with a commercially available kit (Incstar Inc., Stillwater, Minn). The sensitivity of the ACTH RIA was 15 pg/ml.

### In situ Hybridization

CRF mRNA was quantitated in unstressed handled and MS8 male rats at about PND 60–65 using in situ hybridization with antisense riboprobes to CRF as previously described (Makino et al. 1995). The cRNA probe for rat CRF was transcribed according to the manufacturer's instructions (Ambion, Austin, TX) from a 1 kB cDNA insert in pGEM 4 containing the full length coding region of rat CRF (kindly provided by Dr. K. Mayo, Northwestern University and radiolabeled with $^{35}$S-UTP.

### Drugs

DMP696 was synthesized at DuPont Pharmaceuticals (He et al. 2000) and administered 1 h prior to testing by oral gavage as a suspension in 0.25% methylcellulose. Chlordiazepoxide (Sigma) was also suspended in 0.25% methylcellulose and administered orally. Drug volumes were 1 ml/kg. Vehicle groups were administered 0.25% methylcellulose alone.

### Statistics

Results are expressed as the mean and the standard error of the mean. Statistical significance between groups was determined by 2- or 3-way analysis of variance (ANOVA) followed by least squares mean post hoc tests using SuperANOVA and StatView software.

## RESULTS

### Social Interaction Test

In the first experiment, we examined the ability of DMP696 to alter social behavior in adult, male rats that had previously been handled or maternally separated as neonates. We chose 30 mg/kg as a dose of DMP696 which would block about 90% of the CRF type 1 receptors based on ex vivo binding data (He et al. 2000). Behavioral testing of the handled and maternally separated rats began about day 60 of life. Pairs of unfamiliar rats (either two maternally separated or two handled) were placed in an open field and their behaviors scored over a 10-min period. One hour prior to testing, animals received either vehicle or 30 mg/kg DMP696 by oral gavage. The young adult rats that had been previously separated during days 2–10 (the MS8 group) displayed fewer appropriate social interactions compared with the handled animals ($F_{1,36}$=5.1, $p < .03$). DMP696 increased the frequency of social interactions in MS8 as well as in the handled animals ($F_{1,36} = 37.7$, $p < .0001$). (See Figure 2.) The observed decrease in social interac-

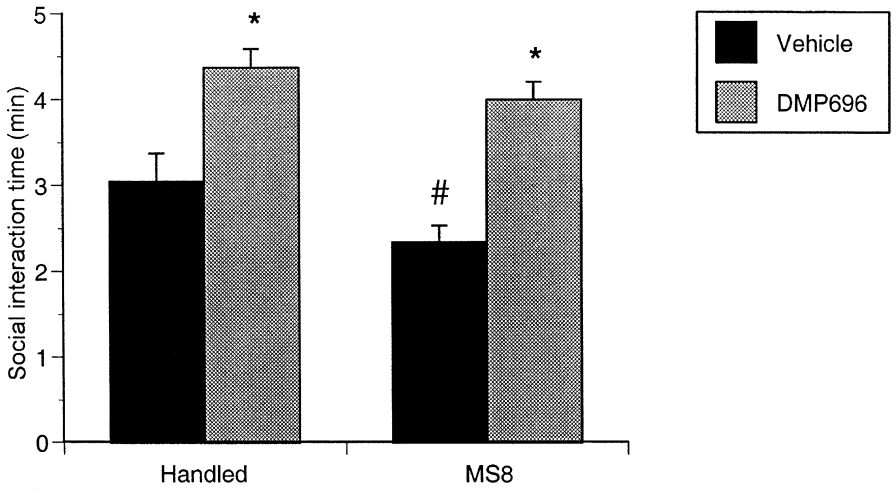



**Figure 2.** Effects of maternal separation and DMP696 (30 mg/kg) on Social Interaction. Results are expressed as the number of minutes the pair of rats spent in typical social behavior during a 10 min observation period. * Significantly different from corresponding vehicle group, $p < .05$ (2-way ANOVA followed by least squares mean analysis). # Significantly different from the handled vehicle group, $p < .05$. n = 10 pairs of rats per group.

tion in the MS8 group was consistent with our general observations that the separated rats vocalized more, were more agitated, and attempted to escape more often than the handled rats. However, there was no effect of separation on adult weights which were similar in the MS8 (321 ± 6 grams) and handled animals (311 ± 8 grams).

### DMP696 Comparison With Chlordiazepoxide In the Elevated Plus Maze

In a second experiment, we compared the effects of DMP696 with a known anxiolytic, the benzodiazepine chlordiazepoxide, in the elevated plus maze. Various doses from 3 to 30 mg/kg were administered. Although 3 mg/kg of DMP696 is an effective dose in defensive withdrawal (He et al. 2000), we have found that 30 mg/kg may be necessary to decrease anxiety-like behavior in normal Sprague Dawley rats in the plus maze (CM Maciag, unpublished observations). Here male rat pups (PND 60–65) were separated and tested in an elevated plus maze 1 h after drug administration. In the vehicle-treated groups, maternally separated animals were less likely to enter the open arms compared with handled animals, although the overall effect of separation on percentage of time spent in the open arms (including the drug-treated groups) was not significant ($F_{1,107} = 2.4$, $p = .12$). See Figure 3. There was however a highly significant overall drug effect ($F_{4,107} = 24.2$, $p < .0001$). Interestingly, there was also an interaction between separation and drug ($F_{4,107} = 3.8$, $p < .01$). DMP696 increased the percentage of time in the open arms only in the MS8 animals. Chlordiazepoxide significantly increased the amount of time spent in the open arms in both handled and MS8 rats. Analysis of the total time in the open arms (as opposed to the percentage of time) gave very similar results with an overall effect of separation which approached significance ($F_{1,107} = 3.7$, $p = .057$), a highly significant drug effect ($F_{4,107} = 21.1$, $p < .0001$), and an interaction between separation and drug ($F_{4,107} = 3.5$, $p = .01$). As expected from the open arm results, DMP696 significantly decreased time spent in the closed arms but only in the MS8 rats, whereas chlordiazepoxide again affected both groups (separation and drug interaction $F_{4,107} = 5.9$, $p = .0002$). See Table 1.

When looking at entries into the open arms instead of time, DMP696 (30 mg/kg) tended to increase the percentage of entries into the open arms but again only in the MS8 group (Figure 3). Lower doses of DMP696 (3 or 10 mg/kg) did not produce significant effects. DMP696 (30 mg/kg) increased the number of open entries made by the MS8 rats but not by the handled group during the 5-min period (see Table 1). For open entries, there was not an overall effect of separation ($F_{1,107} = 1.5$, $p = .22$), but there was a highly significant drug effect ($F_{4,107} = 24.6$, $p < .0001$), and a strong trend toward an interaction between separation and drug effects ($F_{4,107} = 2.3$, $p = .06$). In contrast, DMP696 had no effect on the number of closed entries in either group (see Table 1). Chlordiazepoxide increased open entries in both groups. Curiously, chlordiazepoxide also tended to increase closed arm entries especially in the MS8 group. This would indicate that this dose of chlordiazepoxide was not sedating.

### DMP696 Comparison with Chlordiazepoxide in Suppression of Stress-induced ACTH Release

Previous studies have typically found that adult rats which had been maternally separated as infants secrete more ACTH in response to stressors compared with handled rats (Plotsky and Meaney 1993; Patchev et al. 1997; Ladd et al. 2000). Here we measured plasma ACTH levels 5 min after a single footshock. Although basal (no shock) ACTH levels were slightly higher than one would expect, there was a significant effect of the

footshock to elevate ACTH ($F_{1,97}$=11.5, $p < .001$). Stress induced ACTH levels were significantly higher in the MS8 rats compared with the handled (see Figure 4). There was a significant drug effect ($F_{2,97}$=4.8, $p = .01$) and an interaction between drug and stress ($F_{2,97}$=6.3, $p < .003$) such that both DMP696 and chlordiazepoxide blocked the stress-induced increase in ACTH but only in the MS8 group (see Figure 4). We also measured basal PVN CRF mRNA levels in unstressed PND 65 rats, which were found to be significantly higher in MS8 (12515 ± 2850) versus handled animals (6758 ± 2282) (Student *t*-test, $p < .05$).

## DISCUSSION

The primary finding of this study is that the non-peptide CRF antagonist DMP696 is indeed capable of reducing anxiety-like behavior in adult rats that were maternally separated as pups. This was apparent in both behavioral tests, social interaction and the elevated plus maze. In the handled animals by contrast, DMP696 reduced anxiety in the social interaction test, but it did not produce a statistically significant effect in the plus maze. It could be argued that this failure of DMP696 to produce robust effects in the handled animals is simply

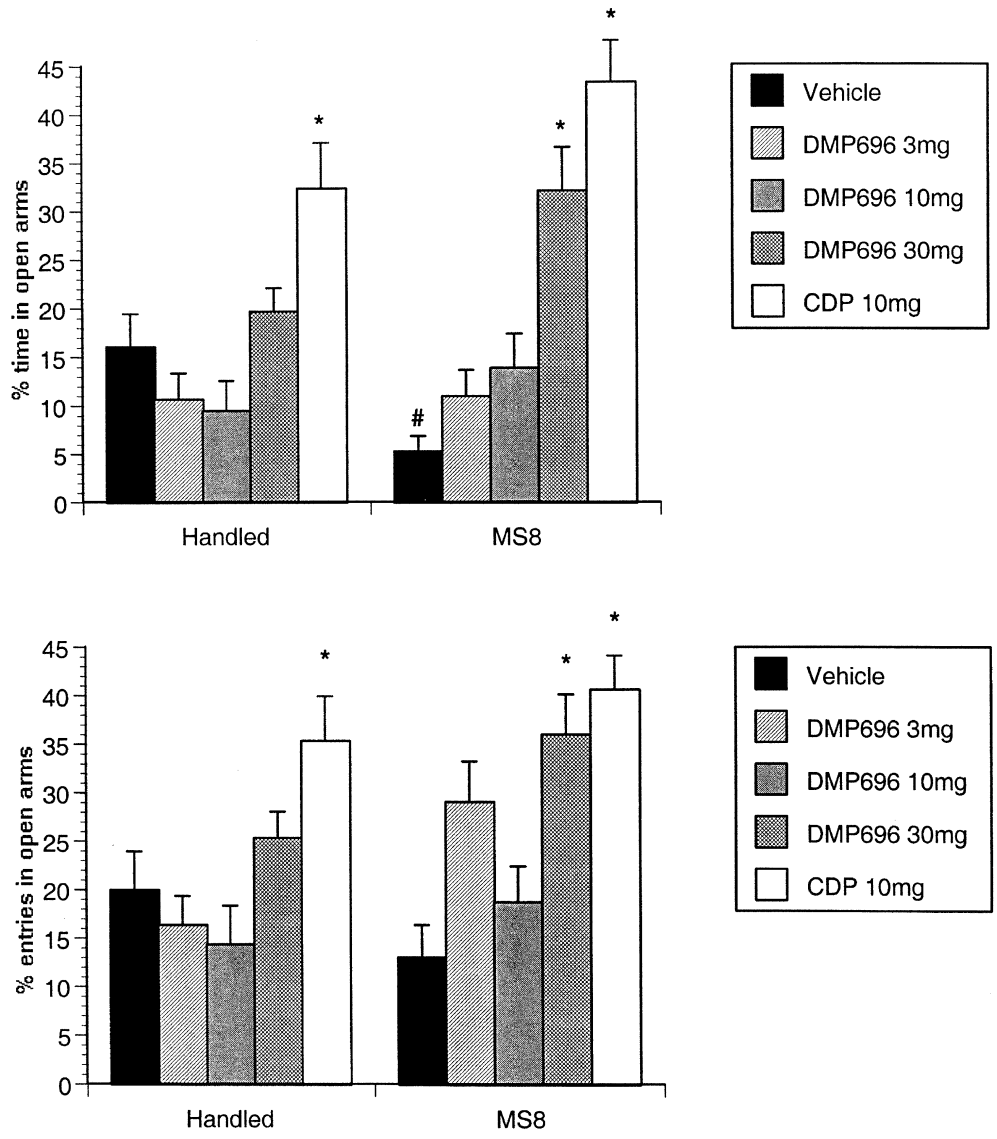



**Figure 3.** Effects of maternal separation and DMP696 on behavior in the elevated plus maze. The effects of the CRF antagonist (DMP696) and chlordiazepoxide (CDP) were examined in adult rats that had been either handled or maternally separated (MS8) during infancy. * Significantly different from corresponding vehicle group, $p < .05$ (2 way ANOVA followed by least squares mean analysis). # Significantly different from the handled vehicle group, $p < .05$. n = 11–13 rats/group.

**Table 1.** Effects of DMP696 and Chlordiazepoxide on Anxiety-related Behavior in the Elevated Plus Maze

| | | Minutes spent in | | Entries into | |
|---|---|---|---|---|---|
| **Group** | **n** | **Open arms** | **Closed arms** | **Open arms** | **Closed arms** |
| MS8 rats | | | | | |
| Vehicle | 12 | 0.18 ± 0.06 | 3.32 ± 0.15 | 1.2 ± 0.3 | 7.2 ± 0.8 |
| DMP696 (3 mg/kg) | 11 | 0.40 ± 0.10 | 3.26 ± 0.14 | 1.8 ± 0.4 | 6.7 ± 0.8 |
| DMP696 (10 mg/kg) | 11 | 0.45 ± 0.13 | 2.74 ± 0.12 | 1.8 ± 0.5 | 7.5 ± 0.5 |
| DMP696 (30 mg/kg) | 12 | 1.18 ± 0.18*** | 2.41 ± 0.14* | 4.3 ± 0.6** | 7.2 ± 0.4 |
| Chlordiazepoxide (10 mg/kg) | 12 | 1.62 ± 0.21*** | 2.00 ± 0.11** | 7.3 ± 0.8*** | 10.2 ± 0.6* |
| Handled rats | | | | | |
| Vehicle | 13 | 0.52 ± 0.12 | 2.67 ± 0.13 | 2.5 ± 0.6 | 8.1 ± 0.5 |
| DMP696 (3 mg/kg) | 11 | 0.36 ± 0.09 | 3.00 ± 0.09 | 1.8 ± 0.4 | 7.9 ± 0.6 |
| DMP696 (10 mg/kg) | 11 | 0.33 ± 0.11 | 3.05 ± 0.10 | 1.5 ± 0.5 | 7.1 ± 0.8 |
| DMP696 (30 mg/kg) | 12 | 0.66 ± 0.08 | 2.75 ± 0.12 | 3.0 ± 0.4 | 8.6 ± 0.5 |
| Chlordiazepoxide (10 mg/kg) | 12 | 1.11 ± 0.20** | 2.12 ± 0.11 | 5.4 ± 0.9** | 9.5 ± 0.9 |

*Significantly different from vehicle group at $p < .05$.
**Significantly different from vehicle group at $p < .01$.
***Significantly different from vehicle group at $p < .001$.

a statistical anomaly or "floor effect", in that the handled rats manifest very little baseline anxiety and hence it may have been difficult to reduce it further. Alternatively, the anxiolytic properties of CRF antagonists may actually be more robust in animals that have a sufficient level of endogenous CNS activation. For example, very recent evidence suggests that another CRF antagonist, R121919 (20 mg/kg), exerts anxiolytic effects in the plus maze in Wistar rats bred for high anxiety but not in those bred for low anxiety (Keck et al. 2001). Similar effects are seen in Syracuse high- and low-avoidance rats (Gupta and Brush 1998). The effects of CRF antagonists in "normal" laboratory rats are made more apparent in the elevated plus maze by prior exposure to stress (Heinrichs et al. 1992). It is noteworthy that in our experiments, we tried not to prestress the animals, so that pre-

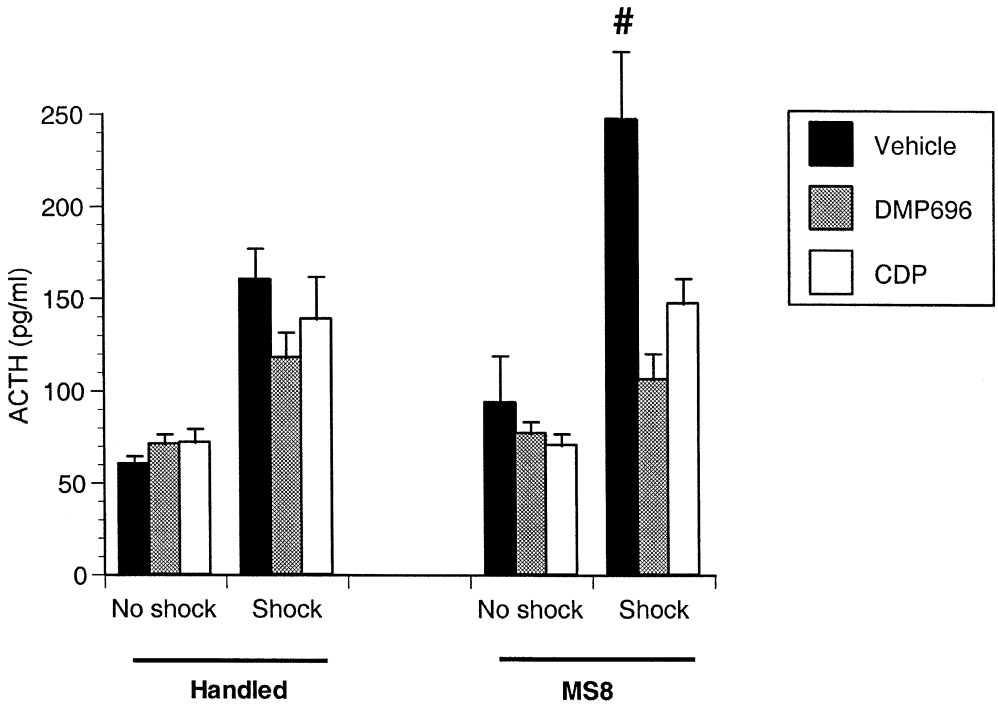



**Figure 4.** Effects of maternal separation and DMP696 on stress-induced ACTH secretion. Plasma ACTH was measured by RIA 5 min after a single footshock in handled and maternally separated (MS8) rats that had been pretreated with vehicle, DMP696 (30 mg/kg) or chlordiazepoxide (CDP 10 mg/kg) 1 h prior to the shock. The unstressed groups received drug but no shock. # Significantly different from all other groups, $p < .05$ (3-way ANOVA followed by least squares mean analysis).

sumably their level of behavioral activation was at baseline just prior to testing. The most likely physiological basis for why CRF antagonists might show more efficacy in compromised animals is differences in endogenous CRF or CRF-R1 expression. Maternal separation is associated with elevated endogenous CRF expression whereas handled animals have decreased CRF levels when compared with non-handled controls (Francis et al. 1999). We confirmed in part this differential expression in the hypothalamic PVN.

While the rat maternal separation model is associated with elevated endogenous CRF expression, it is also characterized by changes in a number of other stress-related neurotransmitters. It is not clear if a CRF antagonist would only block anxiety due to increased endogenous CRF or whether a CRF antagonist may block anxiety mediated indirectly through other mechanisms such as alterations in NE or 5HT, GABA, etc. (DMP696 at 30 mg/kg is not expected to block directly other neurotransmitter receptors based on its excellent specificity for CRF type 1 receptors.) For example, stress-induced elevations in norepinephrine release are greater in maternally separated rats compared with handled animals possibly due to decreased $\alpha_2$ autoreceptors in the locus coeruleus (Liu et al. 2000). In normal laboratory rats, CRF peptide and non-peptide antagonists are capable of blocking LC discharge (Curtis et al. 1994; Schulz et al. 1996). Whether CRF antagonists are able to block the increased norepinephrine release in maternally separated rats has not yet been specifically tested. However it is likely that they would block NE release given the results from normal laboratory animals, and this mechanism of action may contribute to the anxiolytic properties of CRF antagonists.

Another effect of maternal separation is to reduce benzodiazepine receptor expression in the amygdala and locus coeruleus (Caldji et al. 2000). We found that chlordiazepoxide was very effective in suppressing anxiety-like behavior in the maternally separated animals. In contrast to DMP696 however, chlordiazepoxide was also very effective in reducing anxiety levels in the handled animals, which were less anxious than the MS8 group as measured in the elevated plus maze. Not having performed complete dose response curves, we cannot directly compare the potency and efficacy of DMP696 with chlordiazepoxide. However, compared with some CRF antagonists, benzodiazepines are active across a broader range of behavioral tests (Griebel et al. 1998) and in a larger number of rodent strains (Conti et al. 1994). Whether this could be due to better intrinsic efficacy of benzodiazepines or again due to differences in the level of activation of endogenous CRF circuitry among the various behaviors and strains remains to be determined.

This study confirms that maternal separation for 8 h every other day between PND 2 and PND 10 does result in long term changes in the neuroendocrine, endocrine and behavioral profiles in adulthood. We chose this regimen, as opposed to separating the infants for 3 h/day for the first 2–3 weeks of life, because in our hands, it has produced more consistent behavioral and endocrine differences (Gordon 2001). In this particular study, the effects of DMP696 on stress-induced ACTH secretion paralleled its effects on behavior. Thus DMP696 was more effective at blocking stress-induced ACTH in the MS8 animals than it was in the handled group. This could be due to a "floor effect" again or could reflect differences in CRF tone. The observed increase in basal CRF levels of MS8 rats lends support for the latter hypothesis.

Increased stress sensitivity, whether derived genetically or environmentally, is thought to predispose to the development of major depression (Post 1992; Kendler et al. 1995; Brown et al. 1999; Heim et al. 2000). It is possible that early environmental challenges such as maternal separation alter the set point for CRF secretion and thus predispose one to the development of psychopathology. Though it would be incorrect to classify maternal separation as a model of human depression or anxiety disorders, it may not be unreasonable to view it as a state of enhanced vulnerability to stress (Francis et al. 1999). In this regard, it would be of interest to determine if treatment with a CRF antagonist during or soon after maternal separation might prevent the development of depressive and anxiety-like behavior seen in the adult rats. Neonatal administration of the neurosteroid, THDOC, was found to prevent the endocrine and behavioral effects of maternal separation perhaps in part by blocking CRF (Patchev et al. 1997). In preliminary studies, we have established that administration of DMP696, either to the pup or to the lactating dam, significantly attenuates ultrasonic vocalizations induced by separation from the litter. This confirms a recent study with another CRF antagonist CP-154,526 (Kehne et al. 2000). Unfortunately the extra manipulation of drug administration during the separation paradigm has made it difficult to consistently reproduce the behavioral sequelae as an adult and so far prevented us from testing whether DMP696 might block the induction of these effects. Nevertheless, it is worth considering whether CRF antagonists might find utility in altering the progression from trauma to post traumatic stress disorder, anxiety and depression.

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## REFERENCES


Brown J, Cohen P, Johnson JG, Smailes EM (1999): Childhood abuse and neglect: specificity of effects on adolescent and young adult depression and suicidality. J Am Acad Child Adolesc Psychiatry 38:1490–1496

Caldji C, Francis D, Sharma S, Plotsky PM, Meaney MJ (2000): The effects of early rearing environment on the development of GABAA and central benzodiazepine receptor levels and novelty-induced fearfulness in the rat. Neuropsychopharmacology 22:219–229

Conti LH, Costello DG, Martin LA, White MF, Abreu ME (1994): Mouse strain differences in the behavioral effects of corticotropin releasing factor (CRF) and the CRF antagonist alpha-helical CRF9–41. Pharmacol Biochem Behav 48:497–503

Curtis AL, Grigoriadis DE, Page ME, Rivier J, Valentino RJ (1994): Pharmacological comparison of two corticotropin-releasing factor antagonists: in vivo and in vitro studies. J Pharmacol Exp Ther 268:359–365

Francis DD, Caldji C, Champagne F, Plotsky PM, Meaney MJ (1999): The role of corticotropin-releasing factor-norepinephrine systems in mediating the effects of early experience on the development of behavioral and endocrine responses to stress. Biol Psychiatry 46:1153–1166

Gilligan PJ, Robertson DW, Zaczek R (2000): Corticotropin releasing factor (CRF) receptor modulators: progress and opportunities for new therapeutic agents. J Med Chem 43:1–20

Gordon MK (2001): Ph.D. thesis. Newark, DE, University of Delaware

Griebel G, Perrault G, Sanger DJ (1998): Characterization of the behavioral profile of the non-peptide CRF receptor antagonist CP-154,526 in anxiety models in rodents. Psychopharmacology 138:55–66

Gupta P, Brush FR (1998): Differential behavioral and endocrinological effects of corticotropin-releasing hormone (CRH) in the Syracuse high- and low-avoidance rats. Horm Behav 34:262–267

He L, Gilligan PJ, Zaczek R, Fitzgerald LW, McElroy J, Shen HS, Saye JA, Kalin NH, Shelton S, Christ D, Trainor G, Hartig P (2000): 4-(1,3-Dimethoxyprop-2-ylamino)-2,7-dimethyl-8-(2, 4-dichlorophenyl)pyrazolo[1,5-a]-1,3,5-triazine: a potent, orally bioavailable CRF(1) receptor antagonist. J Med Chem 43:449–456

Heim C, Newport DJ, Heit S, Graham YP, Wilcox M, Bonsall R, Miller AH, Nemeroff CB (2000): Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. JAMA 284:592–597

Heinrichs SC, Pich EM, Miczek KA, Britton KT, Koob GF (1992): Corticotropin-releasing factor antagonist reduces emotionality in socially defeated rats via direct neurotropic action. Brain Res 581:190–197

Holsboer F (1999): The rationale for corticotropin-releasing hormone receptor (CRH-R) antagonists to treat depression and anxiety. J Psychiatr Res 33:181–214

Keck ME, Welt T, Wigger A, Renner U, Engelmann M, Holsboer F, Landgraf R (2001): Anxiolytic effect of the $CRH_1$ receptor antagonist R121919 depends on innate emotionality in rats. Eur J Neurosci 13:373–380

Kehne JH, Coverdale S, McCloskey TC, Hoffman DC, Cassella JV (2000): Effects of the $CRF_1$ receptor antagonist, CP 154,526, in the separation-induced vocalization anxiolytic test in rat pups. Neuropharmacology 39:1357–1367

Kendler KS, Kessler RC, Walters EE, MacLean C, Neale MC, Heath AC, Eaves LJ (1995): Stressful life events, genetic liability, and onset of an episode of major depression in women. Am J Psychiatry 152:833–842

Koob GF, Heinrichs SC (1999): A role for corticotropin releasing factor and urocortin in behavioral responses to stressors. Brain Res 848:141–152

Ladd CO, Huot RL, Thrivikraman KV, Nemeroff CB, Meaney MJ, Plotsky PM (2000): Long-term behavioral and neuroendocrine adaptations to adverse early experience. Prog Brain Res 122:81–103

Lehmann J, Feldon J (2000): Long-term biobehavioral effects of maternal separation in the rat: consistent or confusing? Rev Neurosci 11:383–408

Levine S (1957): Infantile experience and resistence to physiological stress. Science 126:1104–1110

Liu D, Caldji C, Sharma S, Plotsky PM, Meaney MJ (2000): Influence of neonatal rearing conditions on stress-induced adrenocorticotropin responses and norepinephrine release in the hypothalamic paraventricular nucleus. J Neuroendocrinology 12:5–12

Makino S, Smith MA, Gold PW (1995): Increased expression of corticotropin-releasing hormone and vasopressin messenger ribonucleic acid (mRNA) in the hypothalamic paraventricular nucleus during repeated stress: association with reduction in glucocorticoid receptor mRNA levels. Endocrinology 136:3299–3309

Mansbach RS, Brooks EN, Chen YL (1997): Antidepressant-like effects of CP-154,526, a selective $CRF_1$ receptor antagonist. Eur J Pharmacology 323:21–26

Meaney MJ, Aitken DH, Sharma S, Viau V, Sarrieau A (1989): Postnatal handling increases hippocampal type II, glucocorticoid receptors and enhances adrenocortical negative-feedback efficacy in the rat. Neuroendocrinology 51:597–604

Meerlo P, Horvath KM, Nagy GM, Bohus B, Koolhaas JM (1999): The influence of postnatal handling on adult neuroendocrine and behavioural stress reactivity. J Neuroendocrinol 11:925–933

Nemeroff CB, Widerlov E, Bissette G, Walleus H, Karlsson I, Eklund K, Kilts CD, Loosen PT, Vale W (1984): Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. Science 226: 1342–1344

Patchev VK, Montkowski A, Rouskova D, Koranyi L, Holsboer F, Almeida OF (1997): Neonatal treatment of rats with the neuroactive steroid tetrahydrodeoxycorticosterone (THDOC) abolishes the behavioral and neuroendocrine consequences of adverse early life events. J Clin Invest 99:962–966

Post RM (1992): Transduction of psychosocial stress into the neurobiology of recurrent affective disorder. Am J Psychiatry 149:999–1010

Plotsky PM, Meaney MJ (1993): Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. Mol Brain Res 18:195–200

Schulz DW, Mansbach RS, Sprouse J, Braselton JP, Collins J, Corman M, Dunaiskis A, Faraci S, Schmidt AW, Seeger T, Seymour P, Tingley FD 3rd, Winston EN, Chen YL, Heym J (1996): CP-154,526: a potent and selective nonpeptide antagonist of corticotropin releasing factor receptors. Proc Natl Acad Sci USA 93:10477–10482

Zobel AW, Nickel T, Kunzel HE, Ackl N, Sonntag A, Ising M, Holsboer F (2000): Effects of the high-affinity corticotropin-releasing hormone receptor 1 antagonist R121919 in major depression: the first 20 patients treated. J Psychiatr Res 34:171–181