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A CCK_A-Receptor Antagonist Administered to the Neonate Alters Mother–Infant Interactions in the Rat

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WELLER, A. AND L. DUBSON. *A CCK_A-receptor antagonist administered to the neonate alters mother–infant interactions in the rat.* PHARMACOL BIOCHEM BEHAV **59**(4) 843–851, 1998.—The importance of the infant's cholecystokinin (CCK) system for eliciting optimal maternal care was examined in 6–9-day-old Sprague–Dawley rats. After administration of either vehicle, CCK-8 (1 or 8 μg/kg) or devazepide (1 mg/kg; a selective CCK_A receptor antagonist), pups were either individually isolated (Experiment 1) or individually reunited with their dam (Experiment 2) and the rats' behavior was observed. When isolated, pups that received devazepide displayed significantly more head-lifting and wall-climbing attempts than vehicle-treated controls, suggesting that endogenous CCK dampens activity. Devazepide-treated rats were found more frequently in proximity with their mothers when reunited with them, and they emitted more ultrasonic vocalizations compared to vehicle controls. Pups treated with 1 μg/kg CCK received less body licking than vehicle controls. In addition, dams hovered and crouched over devazepide-treated pups more than over pups treated with 1 μg/kg CCK. The results suggest that endogenous CCK has a calming, quieting effect in the neonatal pup and that this, in turn, results in less infant–mother attractivity and reduced levels of maternal care. © 1998 Elsevier Science Inc.

Maternal behavior Ultrasonic vocalization Cholecystokinin (CCK) Devazepide Neonatal rats Infant–mother interactions

THE gastrointestinal tract is the largest endocrine gland in the body. Most of the research on the many (over 20) polypeptide hormones secreted from the gut has focused on their effects on digestion, nutrient transport, and metabolism. Behavioral research has focused mainly on cholecystokinin (CCK), a peptide released from mucosal cells in the proximal small intestine. CCK has been shown to suppress feeding in many species, and other behavioral effects have been documented as well, for example, on analgesia, sleep, memory, and sexual behavior (17). Among its diverse physiological effects, CCK promotes growth of the gastrointestinal tract and the pancreas [cf. (13)], induces the release of nutrient-digesting enzymes, and increases the efficiency of insulin-mediated glucose utilization (17,21,57).

CCK plays an important role in coordinating the developing infant's digestion, metabolism, and growth (55,56). Along with other gut hormones, CCK may also contribute to behavioral development of the infant, and help shape the pattern of its interaction with its mother (55,56). Although these behavioral effects could be limited to the realm of ingestion, potential effects may also include nonnutritive aspects of developing infant–mother (and infant–sibling) relations. The current study examines this possibility in rats. Finding evidence for CCK involvement in infant behavior and maternal care in rats would suggest the possibility (for future research) that peptide gut hormones, such as CCK, exert long-term effects on psychosocial maturation in many mammals, including humans.

Research in neonatal rats has shown changes in plasma CCK levels in response to maternal separation and reunion. Plasma CCK was low in pups separated in a group from their mother overnight and high in pups then reunited with a dam for 1 h, when compared with control littermates taken from the nest (and receiving a gastric load of isotonic saline) (64). The particular aspect(s) of deprivation (e.g., nutritional deficit, dehydration, lack of contact, etc.) and of reunion (e.g., milk intake, suckling, maternal odor, warmth and contact, pup licking, etc.) that are responsible for these changes have yet to be elucidated. Preliminary findings in canine puppies further demonstrate increases in plasma CCK levels during sucking and also when the puppies were returned to their littermates

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after a period of separation (56,58). This suggests that the warmth, touch, or odor of the other puppies elicits CCK release in the subjects, even in the absence of sucking or ingestive stimulation.

The peripheral CCK system of the rat is functional in the neonatal period: receptors are abundant and widely distributed in the gut (49) and both exogenously administered and endogenously released CCK reduce intake (50,65) in tests of independent ingestion away from the dam (24). Furthermore, the selective CCK_A receptor antagonist devazepide (16) blocks feeding inhibition induced either by exogenous or endogenous CCK in neonatal rats (53,65).

Intraperitoneal CCK administration (in a noningestive context) has been shown to selectively decrease separation (from siblings and dam)-induced ultrasonic vocalization (USV) in rat pups, without affecting pain threshold or activity levels (60). More recently, endogenous CCK has been similarly implicated in USV reduction: the CCK_A receptor-antagonist devazepide (1 mg/kg), blocked the selective reduction in USV produced by intraoral infusions of milk and corn oil but not sucrose (10). This finding suggests selectivity and demonstrates that devazepide at the dose used does not uniformly induce distress because it did not elevate USV rates in rats quieted by sucrose. Thus, it is reasonable to hypothesize that the neonatal rat's endogenous CCK system may mediate a portion of the stress-alleviation produced by a "companion," by "contact-comfort" and by milk [e.g., (9,26–28,46)]. In this context it is relevant to note that pairing CCK administration with a novel odor CS results in a conditioned odor preference (62). This was found in infants and neonates, but not in weaned or adult rats, suggesting that CCK's positive rewarding function may be confined to early development.

In the nest, the pups and their mother interact reciprocally and symbiotically from parturition to weaning (2,3). Thus, if CCK affects the infant's behavior, as discussed above, it is reasonable to expect that its mother's behavior will be influenced in turn. Examination of this possibility is the major aim of the present study. Research has shown that the profile of maternal behavior directed toward rat pups can be influenced by the pup's hormonal state. In particular, anogenital licking rates can be increased by administering testosterone, dihydrotestosterone, or progestins to the pups (6,38). This may have long-term consequences, because altered levels of maternal pup licking have been found to affect future social play and adult sexual behavior (7,40,48).

The general thesis examined in the present study is that an active and functional CCK system is necessary for adaptive levels of maternal care and for the infant to demonstrate contact-comfort (operationally defined here by reduction in USV). Pups were either individually isolated (Experiment 1) or individually reunited with their dam and their behavior was recorded in detail. Manipulations included: (a) acute increasing of neonatal CCK by exogenous administration, or (b) blocking availability of endogenous CCK by the CCK_A receptor antagonist devazepide (16,35). The hypothesis was that CCK antagonism in the pup would cause a behavioral pattern different from controls, for example, increased pup activity and USV (i.e., reduced infant "calming" or "comfort") and a pattern of maternal care different from controls. CCK administration was expected to produce the opposite pattern of results.

EXPERIMENT 1

Before studying dam–pup interactions we assessed the behavior of individual pups isolated after injection of CCK or devazepide. This experiment was performed to observe potential behavioral changes that treated pups could exhibit in the dam–infant interaction (Experiment 2). Testing was conducted in Experiments 1–3 after about 3.5 h of deprivation, a time frame in which most (but not all) of the milk ingested would have cleared the stomach. This was an attempt to reach moderate (assumed, not measured) baseline levels of endogenous CCK that could allow both the ligand and the antagonist to operate.

METHOD

Subjects

Multiparous Sprague–Dawley rats were mated in our colony at the Developmental Psychobiology laboratory in the Psychology Department at Bar Ilan University. The litters born served as subjects. Lights in the colony were on from 0500 to 1900 h and temperature was maintained between 21–24°C. Pregnant rats were housed individually in polycarbonate cages (38 × 21 × 18 cm) with stainless steel wire lids and wood shavings as bedding material. Newborn litters found between 0800 and 1200 h were designated as born on that day (day 0) and reduced to 10 pups on day 1. Chow and tap water were continuously available in the cage top. Pups of both sexes were tested on postnatal days 6–9 (body weight range = 9.8–22.5 g). Each pup was tested only once, and only one sibling within a litter was allocated to the same treatment group, thus avoiding the "inflated-n problem" resulting from "litter effects" (1,67). In Experiment 1, 12 litters were tested ($n = 4$ pups/litter).

Treatments

The subjects were divided into four groups, according to the particular treatment. In a within-litter design, pups received an intraperitoneal injection (2 ml/kg b.wt.) of either: 1) devazepide, a specific antagonist of CCK_A receptors (a gift of Merck Co., West Point, PA) dissolved to make a final solution of 1 mg/kg b.wt. in a vehicle containing components similar to those used by (19): 9.5% ethyl alcohol and 4.5% carboxymethyl cellulose (CMC, Sigma) in distilled water. 2) CCK-octapeptide (1 μg/kg b.wt; Sigma), dissolved in isotonic (0.9%) saline. 3) CCK-octapeptide (8 μg/kg b.wt.; Sigma), dissolved in isotonic saline. 4) Control: isotonic saline (60% of the vials) or the above-detailed vehicle, containing alcohol, CMC, and water (40% of the vials). (Preliminary tests suggested no difference in the effects of these two solutions.) The control vials were chosen at random in each litter.

All solutions were stored at −80°C in vials prepared for one pup each, to be defrosted and vortexed just before injection. To attain "blind" treatment and testing, vials were coded.

Test Procedure

All pups from each litter were taken from the nest and dam in the morning (0830–1000, eight litters; 1030–1300, four litters). At this point it was noted if the litter was nursing. The pups were weighed and runt(s) (extremely light) pups were excluded. Four of the remaining pups (two male, two female) were chosen randomly, weighed, and marked, after which all the pups were placed together in a small container in a humid and warm (33°C) incubator. Three to 3.5 h later, each of the four experimental subjects received an intraperitoneal injection of one of the four solutions (order was counterbalanced between litters) and was returned to the litter for 15 min.

Next, each pup was removed individually from the incubator and placed in a clean clear polycarbonate cage (38 × 21 × 18 cm) at room temperature (mean ± SEM = 23.6 ± 0.49°C), for a 6-min observation session. A grid of 2 × 3 squares (10^2 cm each) was placed under the cage, to allow counting of line crossings. The measures recorded were employed after preliminary tests, in following with (26,28,60). They included the number of line-crossings, head lifting, climbing attempts, bouts of self grooming episodes, rolling over, pivoting (180° turns), twitching/shivering, and bouts of USV (transduced by a minibat detector (QMC Instruments, model QMI) set at 45(±5) kHz). Seven of these eight measures were used for data analysis. Because occurrence of twitching/shivering was rare, this measure was excluded from the analysis.

Statistical Analysis

The behavior of the four groups was compared by Kruskal–Wallis (K-W) tests performed on each of the seven measures. This was followed by Mann–Whitney two-sample tests comparing each of the experimental groups (devazepide, CCK 1 or 8 μg/kg) with the vehicle control group.

RESULTS AND DISCUSSION

There was a significant difference between the four groups in their rank scores on head lifting (K-W $\chi^2 = 13.40$, $p < 0.01$), wall climbing (K-W $\chi^2 = 15.82$, $p < 0.01$), and rolling over (K-W $\chi^2 = 9.33$, $p < 0.05$). Further pairwise comparisons showed that the group that received devazepide displayed significantly more head lifting ($Z = 2.48$, $p < 0.05$) and attempts of wall climbing ($Z = 2.65$, $p < 0.01$) than the vehicle control group. The group difference on rolling over ($Z = 1.83$, $p < 0.062$) was not statistically significant. The behavior of the two groups treated with CCK did not differ from controls. The mean number of behaviors observed in each group are presented in Table 1.

These results, showing greater (vertical only) activity in the group with blocked CCK receptors, suggest that endogenous CCK functions in the (opposite) direction, i.e., calming the pup. Similarly, (exogenously administered) CCK has been shown to induce resting and reduce exploration in adult rats and mice (18,37). In the current study, activity changes were not noticed in response to exogenous CCK administration, as in previous research with rat pups (60). Nevertheless, the suggestion derived from the current results (with devazepide) that endogenous CCK has a calming effect is in general accordance with previous findings that exogenously administered and endogenously released CCK reduce levels of distress vocalization (10,60).

Devazepide did not significantly increase USV in the present experiment, in otherwise untreated isolated pups. This negative finding does not necessarily contradict the report of Blass and Shide (10) that devazepide increased USV in isolated rat pups that received quieting (and possibly CCK-releasing) intraoral infusions of milk or corn oil. In contrast, the testing conditions in the current Experiment 1 did not contain such a putative CCK-releasing stimulus. The failure of exogenous CCK (1 μg/kg) to reduce USV in isolated 6–9-day-old pups in the current study contrasts with a previous report in 11-day-old rats (10). This may be accounted for by the age difference and other procedural differences between the two studies, for example, in deprivation duration and ambient temperature at testing.

EXPERIMENT 2

The differential pattern of activity displayed by the pups in the treatment groups studied in Experiment 1 could possibly affect the manner in which their dam will treat and interact with them. Experiment 2 explored this possibility. In this experiment, the interaction between the dam and an individual pup returned to her was studied. This paradigm provided a high level of control, the ability to measure individual USV, and the power of a within-litter treatment design. The behaviors observed in this condition are dominated by the dam; therefore, we focused on maternal behaviors in detail.

METHOD

Subjects and Treatments

Twenty-two multiparous Sprague–Dawley rat dams and their 6–9-day-old pups (body weight range = 9.29–28.80 g) were the subjects in this experiment ($n = 4$ pups/litter). They were raised and maintained as described above.

Test Procedure and Measures

On the test day the pups were treated in a manner identical to that described above, including deprivation period, injec-

TABLE 1

MEAN (SEM) NUMBER OF BEHAVIORS OBSERVED IN RAT PUPS (IN A 6-MINUTE-TEST) AFTER ADMINISTRATION OF VEHICLE, CCK, OR DEVAZEPIDE

		Vehicle	CCK (μg/kg)		Devazepide (mg/kg)
	Dose	0	1	8	1
Behaviors	*n*	(12)	(12)	(12)	(12)
Line crossing		1.50 ± 0.78	1.33 ± 0.78	1.25 ± 0.59	3.08 ± 1.33
Head lifting		2.92 ± 0.60	2.75 ± 0.85	1.83 ± 0.47	6.08 ± 0.93*
Climbing		0.66 ± 0.35	0.92 ± 0.51	0.17 ± 0.11	2.42 ± 0.66†
Self-grooming		0.58 ± 0.29	0.17 ± 0.11	0.25 ± 0.25	1.17 ± 0.46
Rolling over		0.16 ± 0.11	0.33 ± 0.19	0	1.42 ± 0.74
Pivoting		0.75 ± 0.33	0.33 ± 0.19	0.67 ± 0.40	1.42 ± 0.43
USV		9.33 ± 2.20	7.75 ± 2.42	5.33 ± 1.38	13.92 ± 4.43

Between-group comparisons are based on nonparametric tests.
*$p < 0.05$, significantly more than the vehicle-control group.
†$p < 0.01$, significantly more than the vehicle-control group.

tions, and 15-min interval until testing. Their dams waited during this period in a cage with clean wood shavings, in which they established a "nest" area. Next, each pup was removed individually from the incubator and placed with its dam, for a 10-min observation session. The time of onset and conclusion of the following behaviors was recorded: carrying pup in mouth, retrieval of pup to "nest," licking pup's body, anogenital licking, sniffing pup, hovering over pup, crouching with pup attached to nipple, nestbuilding, self-grooming (dam), and bouts of USV (pup). This set of behaviors was selected after preliminary tests, in following with (7,32,39,48,51).

Nineteen measures were derived from this raw data for analysis. These included: total number of occurrence of each of the above behaviors (10 measures); total time devoted to each of the following behaviors: licking pup's body, anogenital licking, sniffing pup, hovering over pup, crouching with pup attached to nipple ("nursing"), nest building, and self-grooming (7 measures); latency to first retrieval and number of episodes of close proximity/contact (0–1 cm) between dam and pups (2 measures).

After the observation session the pup was removed from the test cage and the next subject (a differently treated sibling) was introduced to the dam's cage for observation. In this manner, all four pups were tested with their dam, with testing order counterbalanced between litters and experimenter "blind" to treatment group, as above.

Statistical Analysis

The 19 measures were first subjected to factor analysis, with varimax rotation. Seven factors resulted, accounting for 71% of the variance. They represent: 1) hovering over (with or without pup attached to nipple), USV and dam–pup proximity (17% variance); 2) nest building (13%); 3) sniffing (10%); 4) anogenital licking (10%); 5) pup–body licking (8%); 6) self-grooming (6%); 7) retrieval and carrying pup (6%). Five of the 19 measures had moderate to high loadings (>0.45) on the first factor; two measures displayed >0.45 loadings on each of the remaining six factors (see Table 2).

The behavior of the four groups was compared by one-way multivariate analysis of variance (MANOVA), performed on the factor scores of the above seven factors. This was followed by one-way univariate ANOVA for each of the factors. For a factor on which a significant between-group effect was found, one-way ANOVA examined separately group differences in the specific behaviors that loaded moderately high or high (loading >0.45) on this factor. Significant ($p < 0.05$) main effects were further examined by Duncan's multiple range test ($p = 0.05$). For exploratory purposes, all significant pairwise comparisons resulting from Duncan's test are reported.

The within-litter design utilized in this study is most powerful when the results are analyzed by within-litter statistics, such as within-litter repeated-measures ANOVA (67). This, however drastically reduces the degrees of freedom to the number of litters minus one. In the current experiment, we chose instead to perform between-group (rather than within-litter) analyses, because of the multiple measures assessed. This allowed the (essential) omnibus MANOVA followed by ANOVA approach described above.

RESULTS AND DISCUSSION

The groups differed significantly overall on the seven factors, $F(21, 230) = 2.4$, $p < 0.01$. Examination of group differences on each factor separately showed a significant difference on the first factor "Hovering, USV, and proximity," $F(3, 84) = 7.1$, $p < 0.001$, and on the fifth factor "pup–body licking," $F(3, 84) = 3.3$, $p < 0.05$. There was no significant difference on the remaining factors. Group mean factor scores are shown in Table 3. Post hoc Duncan's tests ($p = 0.05$) showed

TABLE 2

FACTOR LOADINGS OF THE MEASURES OBSERVED IN EXPERIMENT 2

	Factor Number						
Behaviors	1	2	3	4	5	6	7
Hovering	**0.49**	0.04	0.13	−0.24	0.09	−0.02	0.09
T* Hovering	**0.82**	0.04	−0.03	−0.10	−0.01	−0.09	0.11
Nursing	**0.73**	−0.03	−0.07	0.13	−0.21	0.06	−0.30
T* "nursing"	**0.73**	−0.01	−0.02	0.01	−0.19	0.10	−0.18
USV	**0.77**	−0.01	−0.04	−0.01	−0.01	−0.21	0.13
Proximity	**0.48**	0.44	−0.19	0.37	−0.01	0.16	−0.16
Nest building	0.02	**0.87**	0.16	0.16	0.03	0.01	0.10
T* Nest build.	0.05	**0.90**	0.04	−0.01	0.09	−0.03	0.02
Sniffing	−0.03	0.09	**0.97**	0.03	0.07	−0.06	−0.04
T* Sniffing	−0.01	0.08	**0.98**	0.03	0.04	−0.04	−0.04
AGL†	−0.04	−0.15	0.13	**0.86**	0.12	−0.01	0.10
T* AGL	−0.10	−0.02	−0.04	**0.91**	−0.00	0.08	0.05
Body-licking	−0.14	0.15	0.04	−0.03	**0.84**	0.06	0.14
T* Body-lick.	−0.09	−0.02	0.07	0.13	**0.85**	−0.15	−0.08
Self-grooming	−0.11	−0.05	0.07	0.11	−0.02	**0.81**	0.08
T* Self-groom.	0.01	0.04	−0.17	−0.04	−0.06	**0.84**	−0.04
Retrieval	−0.03	0.00	−0.04	0.16	0.00	0.07	**0.80**
Latency to ret.	−0.18	0.25	0.11	−0.06	−0.26	−0.04	−0.39
Carrying pup	−0.13	0.41	0.02	−0.11	−0.04	−0.08	**0.63**

T* = Total time.
AGL† = Anogenital licking.

that on factor 1 the group that received devazepide had factor scores that were significantly higher than all other three groups. On factor 5, the devazepide-treated group had scores significantly higher than both CCK-treated groups but not from the control group; the group treated with 1 μg/kg CCK had a significantly lower score on this factor compared to the control group. Thus, devazepide-treated pups were apparently observed to be more vocal, and their dam was closer to them and hovered over them (factor 1), compared to all other groups. Furthermore, dams licked their devazepide-treated pup's bodies more (factor 5) than they licked their CCK-treated pups and CCK (1 μg/kg)-treated pups received less licking than controls ($p < 0.05$).

Examination of the eight measures loading high on factors 1 and 5 (Fig. 1) revealed significant between-group differences in 1) proximity to the dam, $F(3, 84) = 8.0$, $p < 0.01$; 2) hovering over the pups, $F(3, 84) = 2.9$, $p < 0.05$; and 3) pup USV, $F(3, 84) = 4.3$, $p < 0.01$. Group differences in total time hovering over pups, $F(3, 84) = 2.3$, $p < 0.085$, number, and duration of "nursing" (i.e., crouching with pup–nipple contact) episodes ($p > 0.1$), number, $F(3, 84) = 2.3$, $p < 0.086$, and duration, $p > 0.1$, of body licking episodes were not significant. Post hoc Duncan's tests ($p = 0.05$) showed that dams interacting with pups that received devazepide were observed more often closer to their pups (compared to all other groups) and devazepide-treated pups vocalized more (than all the other three groups). The post hoc comparison further showed that compared to the group treated with 1 μg/kg CCK, devazepide-treated pups were observed more frequently hovering over their pups and "nursing" them, and both behaviors were observed for longer durations ($p < 0.05$ on all four measures). Finally, pups treated with 1 μg/kg CCK received significantly less body licking than vehicle controls (Fig. 1).

The results of Experiment 2 show that blockade of CCK_A receptors with devazepide results in pups that vocalize more and are found more frequently in proximity with their dam after separation and reuniting. Devazepide may have produced this pattern of behavioral changes by several paths. For example (devazepide—directly induced), increased vocalization may have attracted the dam (4,45), thus increasing proximity, contact, and the consequent licking and hovering over, in the natural sequence (54). Alternatively, the dams could have been attracted to the devazepide-treated pups by nonauditory stimuli (e.g., visually by their conceivably greater activity levels as seen in Experiment 1 or by a devazepide-elicited olfactory signal), and their subsequent handling, licking, and hovering over the pups may have then (secondarily) induced increased levels of pup vocalization (44,46). Thus, more research is still needed before we can decide if endogenous CCK mediates differential USV directly in the pup–dam interaction setting.

The devazepide-induced USV increase in this experiment is interesting also in contrast to the lack of a significant effect on USV in Experiment 1. The pups studied while reunited with their dam in Experiment 2 (in the control and CCK-treated groups) vocalized much less than the pups isolated in a clean cage in Experiment 1 (compare USV data in Fig. 1 and Table 1). This difference could be explained either by maternal absence/presence or by the pup's thermoregulatory challenge: the presence of bedding and a warm dam in Experiment 2 would be expected to reduce USV compared to isolation on a bare plastic surface in Experiment 1. Devazepide-treated pups were the exception: they displayed similar levels of vocalization when reunited with their dam (Experiment 2) as when isolated (Experiment 1). We suggest that the stimulation of maternal reunion may have elicited endogenous CCK secretion in the pups (as reported by Weller et al., 1992, after prolonged separation and reunion). This could have provided an elevated level of peptide against which devazepide effectively increases USV, as found also after oral infusions of milk and corn oil (10). In contrast, acutely isolated pups, as in Experiment 1, may not have much available CCK, explaining the relative inefficacy of devazepide in affecting USV. According to this speculation, the effect of devazepide on (vertical) activity (Table 1) found in Experiment 1 may be mediated by a different, lower affinity CCK_A receptor population (41,59).

As in Experiment 1, exogenous CCK treatment was generally ineffective. However, the current experiment's results produced some important exceptions, all in the hypothesized directions. The lower dose used, 1 μg/kg CCK, significantly reduced the amount of body licking that the pups received from the dam, compared to controls. Furthermore, pups receiving this dose of CCK received less maternal care than devazepide-treated pups on four measures, all related to hovering and crouching ("nursing"). The efficacy of the low (and not the higher) dose is in accordance with previous findings

TABLE 3

MEAN (SEM) FACTOR SCORES IN EXPERIMENT 2 AFTER ADMINISTRATION OF VEHICLE, CCK, OR DEVAZEPIDE

		Vehicle	CCK (μg/kg)		Devazepide (mg/kg)
	Dose	0	1	8	1
Behaviors	*n*	(22)	(22)	(22)	(22)
Factor 1		−0.08 ± 0.15	−0.36 ± 0.10	−0.32 ± 0.11	0.75 ± 0.32*†
Factor 2		−0.21 ± 0.16	−0.03 ± 0.24	−0.05 ± 0.20	0.29 ± 0.23
Factor 3		0.02 ± 0.24	0.04 ± 0.22	0.08 ± 0.21	−0.14 ± 0.19
Factor 4		−0.05 ± 0.22	0.07 ± 0.19	−0.15 ± 0.18	0.27 ± 0.26
Factor 5		0.28 ± 0.22	−0.38 ± 0.19*	−0.25 ± 0.20	0.35 ± 0.21†
Factor 6		0.20 ± 0.25	−0.04 ± 0.23	0.00 ± 0.12	−0.15 ± 0.23
Factor 7		−0.27 ± 0.17	0.03 ± 0.18	−0.10 ± 0.24	0.34 ± 0.25

Between-group comparisons are based upon Duncan's multiple range tests ($p < 0.05$).
*Significantly different from the vehicle-control group.
†Significantly different from both CCK-treated groups.

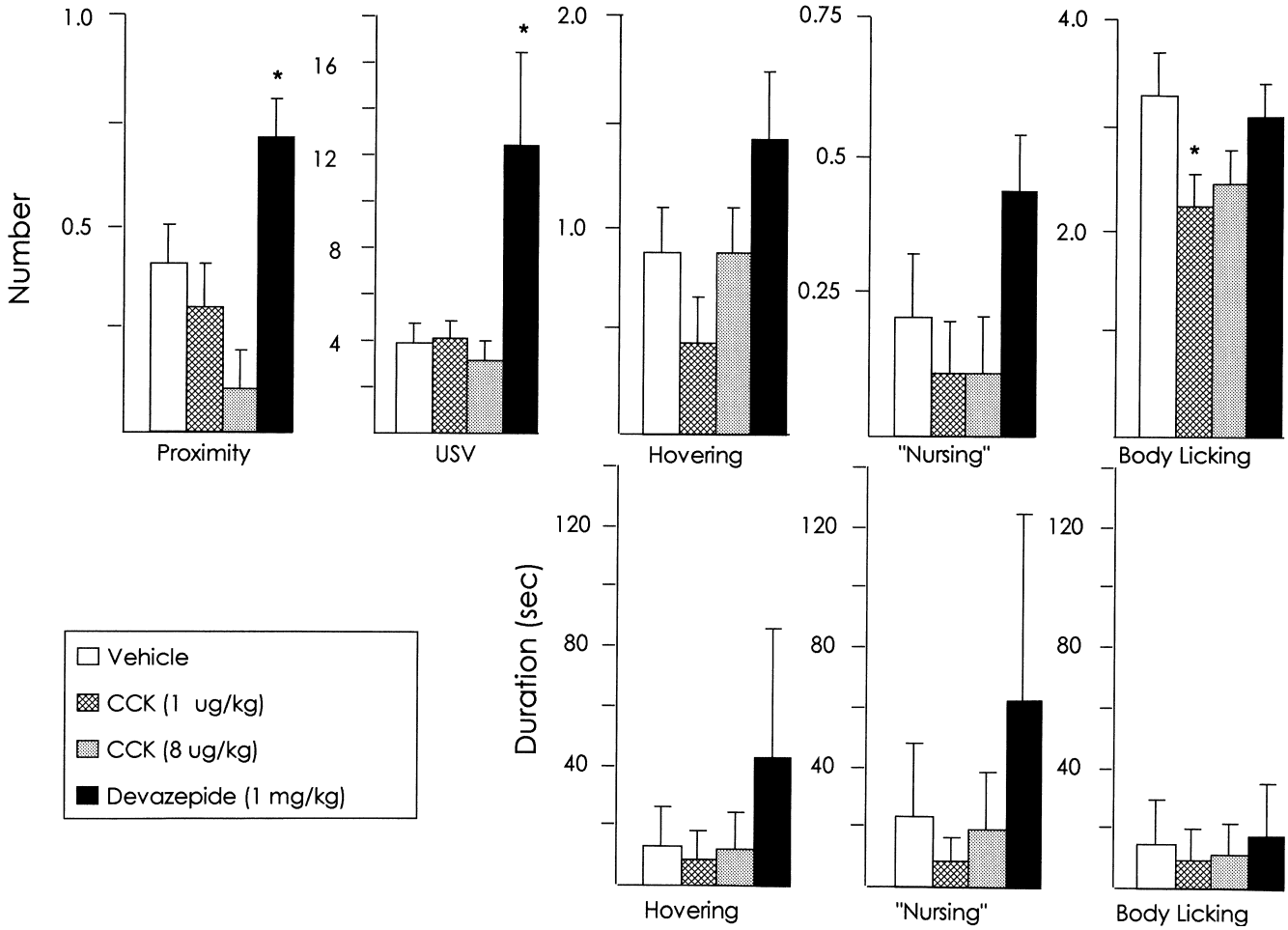

FIG. 1. Mean (+SEM) number and duration of behaviors (comprising factors 1 and 5) of rat dams (and pup USV) in a 10-min test after administration of vehicle, CCK, or devazepide to the pup. *Significantly different from the vehicle-control group (Duncan's test, $p < 0.05$). Significant differences between the experimental groups are described in the text (Results—Experiment 2).

on different measures (60,62). The reduced levels of body licking after CCK administration is reminiscent of the reports of reductions in anogenital licking of pups that received other hormones (6,38). The devazepide–CCK difference found in hovering is consistent with the other significant findings.

EXPERIMENT 3

The major findings of the previous experiments were in differences between the devazepide-treated group and the control group. In addition to the absence of devazepide, 60% of the control injections differed from the devazepide injection in one other aspect: they contained saline, not the (alcohol and CMC and water) vehicle. The possibility still existed that the pattern of results found could be accounted for by the difference in vehicle solution composition. Even though small-scale preliminary experiments had suggested that the two control treatments (saline and vehicle) did not produce different behavioral effects, we examined this issue formally in Experiment 3, to allow clearer interpretation of the results of the first two studies. In a within-litter design, pups received either saline or vehicle and were subsequently tested either according to the procedure described in Experiment 1 or in Experiment 2.

METHOD

Subjects and Treatments

Six multiparous Sprague–Dawley rat dams and their 6–9-day-old pups were the subjects in this experiment ($n = 8$ pups/litter). They were raised and maintained as described above.

Test Procedure, Measures, and Statistics

The test procedure was similar to that described above (deprivation, injection, and test). In the current experiment four pups per litter received vehicle and the other four received saline. Of these, four pups (two saline-and two vehicle-injected) were tested alone (Experiment 3a), as in Experiment 1, and the other four were tested with their dam (Experiment 3b), as in Experiment 2 ($n = 2$ pups/treatment/litter). The data were analyzed separately for the two experimental procedures ($n = 12$ pups/treatment group/experiment).

The behaviors recorded in Experiment 3a were seven of the eight recorded in Experiment 1, with the exception of self-grooming. Nine of the 10 behaviors observed in Experiment 2 were recorded in Experiment 3b; nest building was not recorded. Therefore, 17 of the 19 measures analyzed in Experi-

ment 2 were analyzed in Experiment 3b (excluding number and duration of nest building episodes).

The two treatment groups were compared by within-litter analysis. To address the "inflated-n problem" presented by "litter effects" (67), litter means (per treatment group) were calculated first for each variable and the two treatment groups were then compared on the basis of these means ($n = 6$ litter means/group). Paired (within-litter) *t*-tests were performed to uncover group differences occurring on any single measure. According to Zorrila (67), this approach to analysis of our data is both powerful and sensitive.

RESULTS AND DISCUSSION

No significant between-group effects were found ($p > 0.05$) in Experiments 3a and 3b. We conclude that the pattern of results produced by devazepide in Experiments 1 and 2 may not readily be accounted for by the vehicle solution in which it was administered.

GENERAL DISCUSSION

The present findings suggest that endogenous CCK has a pacifying, calming, quieting effect in the week-old pup and that this, in turn, results in less infant–mother interaction and reduced levels of maternal care. This is inferred from the (vertical) hyperactivity, vocalization, and increased maternal proximity observed in pups treated with the highly specific CCK_A receptor antagonist devazepide. Dams also hovered and crouched more over devazepide-treated pups than over pups treated with 1 μg/kg CCK. Pups receiving this dose of exogenous CCK received less body licking from their dam than vehicle controls.

High, pacifying levels of CCK may be the rule in the litter after the termination of the meal. In fact, we found in a previous study that plasma CCK levels were dramatically increased in pups reunited with a lactating dam for an hour after overnight pup–dam separation (64). In nature, this high CCK level could allow the dam to leave the litter after nursing [to lose heat and to forage; (33)], while the pups are relatively sedated and unlikely to stray from the nest or vocalize and attract predators [as suggested by Blass; (10,11)].

The current findings suggest that blockade of the pups' CCK_A receptors by devazepide resulted in changes in some of the maternal behaviors directed towards them, in the hypothesized direction. But what stimuli were devazepide-treated pups presenting to their dams that resulted in this altered, increased, and close treatment? Three sensory modalities are most likely implicated: vision, audition, and olfaction. Experiment 1 suggests that the pups were moderately more active, and this hyperactivity (if exhibited in the presence of the dam; pup activity was not examined in Experiment 2) may have elicited increased maternal attention and care via visual (or tactile) stimuli. The findings in Experiment 2 further add the elevated level of ultrasonic vocalization of the pups, which has previously been shown to be increased by devazepide [in pups with reduced baseline vocalization following oral infusions of milk or corn oil (10)]. Lactating rats orient toward pups emitting USV and tend to retrieve the pups when found (4,45), apparently responding differentially to males and females according to vocalization levels (42). Another sensory system that may be involved is olfaction; administration of other hormones (e.g., testosterone, dihydrotestosterone, or progestins) to rat pups (6,38) has been shown to alter their odor, thus affecting levels of pup (anogenital) licking by their dams (6,40,48).

The effects of devazepide in this study were focused, but relatively limited. Thus, the findings suggest a role for endogenous CCK in the rat pup, together with other hormones, for example, steroids (6,38), and neurochemicals, for example, opioids (30,31,52), that affect infant distress and infant–mother interactions.

It is possible that individual differences among pups in their relative levels of endogenous CCK account for a portion of the differences in intensity and quality of infant–mother interactions. We will speculate on three aspects and implications of this prospect.

First, Moore, co-workers, and others have shown that a testosterone-affected odor in pup urine induces rat dams to direct more anogenital licking to male than to female pups, and that this differential licking results in future differential levels of self-grooming and sexual behavior in the offspring (6,7,38–40,48). It is similarly possible that within- and between-litter differences between pups in levels of endogenous CCK elicit subtle differences in the quality of maternal care, and that this can have long-lasting behavioral–developmental implications. It is relevant to note a recent report that behavioral profiles of rat pups, including their relative ability to attract the mother to retrieve them, predicted the rat's behavioral profiles as adults in a "difficult food supply social situation" (20).

Second, rat pups can learn an association between a novel odor and exogenously administered CCK after one pairing, resulting in an increased odor preference and in conditioned odor suppression of intake and vocalization (61–63). It may, therefore, be possible that individual differences between pups in endogenous CCK levels will not only affect the quality of interactions with the dam (as suggested by the current findings) but also the ability of the pup to learn new associations from these interactions.

Third, vocalization of pups separated from the nest and the quieting following reunion with the mother have been viewed as an index of infant–mother attachment [e.g., (12,25)] and as an animal model of human anxiety [e.g., (14,15,29)]. In this context, the findings described above regarding CCK suggest that the infant's CCK system may partially mediate its affective state: the degree of separation anxiety and the readiness to be comforted by the mother's return, at least in rats. This has implications for understanding the biological (and peptidergic) basis for socioemotional development: CCK may be implicated together with other peptides [e.g., oxytocin and beta-endorphin; (5,8,43,66) in shaping the individual's future social and affective profile and therefore in the emerging abilities to cope with stressful events.

Although the simplified mother–infant situation examined in this study provided important preliminary information on the involvement of CCK, the conclusions may not be readily generalizable to other, more typical, dam–pup interactive situations. It is possible that the pattern of CCK's influence on infant–mother interactions observed in this study is unique to the situation where one pup is separated and returned to its dam. If so, devazepide may have a different influence differently (and endogenous CCK may, consequently, have a different role) in the interaction of a group or whole litter with their dam, and in the natural nest situation without the perturbations of separation and reuniting. Further studies are required to examine the generality of our findings.

The ability to generalize from the current results is also dependent upon the degree of physiological relevance of the manipulations. Exogenous CCK was administered in this study in addition to endogenous levels. Thus, this (relatively ineffective) treatment can be regarded as above the natural,

physiological range. Nevertheless, we note that the lower dose used, 1 μg/kg, is considered relatively low and only moderately supraphysiological, and this dose has produced behavioral effects in neonatal pups in previous studies [e.g., (60,62)] and was moderately effective in Experiment 2. Further studies should examine the effects of increased endogenous CCK secretion in the pup (elicited by nutrients, trypsin inhibitor, or otherwise) on pup–dam interactions.

Devazepide, at the dose range used in this study, effectively and selectively blocks CCK_A receptors, which are abundant in the periphery and exist in many sites in the CNS, and not brain-type (CCK_B) receptors or receptors of many other neurochemicals (35). Because devazepide can cross the blood–brain barrier (47), the blockade attained is of one receptor type, but not only peripheral. Thus, we cannot rule out the possibility that some of the current effects were mediated centrally.

In conclusion, the present findings suggest that the gut hormone CCK may play a mediating role in infant development and infant–mother interactions by influencing infant behavior and attractivity and in following maternal care. These findings join others suggesting a role for maternal CCK in mediating (the maintenance of) maternal behavior (22,34,36), and reports of developmental changes in other peptide gut hormones, for exmaple, somatostatin, secretin, and gastrin [e.g., (23,58)]. Overall, this growing literature supports the role of gastrointestinal hormones in coordinating and synchronizing infant–mother development, as suggested by Uvnas-Moberg (55,56).

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