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Ultrasound Vocalization Is Not Related to Corticosterone Response in Isolated Rat Pups

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KLINT, T. AND G. ANDERSSON. *Ultrasound vocalization is not related to corticosterone response in isolated rat pups.* PHARMACOL BIOCHEM BEHAV 47(4) 947–950, 1994.—Isolated from their mother, rat pups respond with changes in ultrasound vocalization (USV), a paradigm that can be used as a test for a large range of anxiolytics. Because the relation between corticosterone (CORT) and putative stress responses like USV is not clear, we examined the effects of the benzodiazepine drugs chlordiazepoxide and diazepam vs. the nonbenzodiazepine drugs buspirone and 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) on USV and plasma CORT concentrations. All drugs caused a dose-related decrease in USV, but only buspirone and 8-OH-DPAT induced a dose-related increase in CORT. We suggest that the seemingly paradoxical effects of buspirone and 8-OH-DPAT, that is, the decrease in USV and the concomitant increase in plasma CORT, are due to the fact that these two drugs act as full agonists at both pre- and postsynaptic 5-HT_{1A} receptors. Our results indicate that, when measured as an increase in the activity of the pituitary adrenocortical axis, the stress response can be interpreted in markedly different ways, depending on whether the increased activity is elicited by an environmental stressor or by pharmacological manipulation.

Ultrasound Corticosterone Buspirone Chlordiazepoxide Diazepam 8-OH-DPAT Rat pups

WHEN isolated from their mother and littermates, rat pups emit ultrasound vocalization (USV) in the frequency range 30 to 50 kHz (10,22). This behavior is most prevalent during the second week postpartum, and at that time the rat pups also exhibit intense changes in USV in response to both environmental and pharmacological manipulations (12,13). The discovery that clinically relevant anxiolytics affect rat pup USV, a paradigm that parallels separation anxiety in human infants and other young mammals, was the original incentive for using USV as an animal model of anxiety in psychopharmacological research (2,7,15,20). Subsequent experimantation has shown that USV due to isolation is attenuated by benzodiazepine anxiolytics (5,20,21) and that the nonbenzodiazepine anxiolytics buspirone and ipsapirone also inhibit USV in rat pups (9).

In general, plasma corticosterone (CORT) levels in adult rats are increased by manipulations associated with handling or by experience of a novel environment. In many models of anxiety, such stress-induced increase in CORT can be attenuated by pretreatment with benzodiazepines (6,18), whereas pretreatment with ipsapirone induces both an anxiolytic-like effect (3) and an activation of the pituitary–adrenocortical axis (16). Considering young rats (3 to 14 days old), only small or no elevations of plasma CORT are seen in response to various stressors (23,27).

The causal relation between the USV and CORT responses in young pups is not fully understood. Therefore, the present study was initiated to determine the effects of pharmacological treatments with benzodiazepine and nonbenzodiazepine anxiolytics on USV and plasma CORT concentrations in 10-day-old rat pups.

METHOD

Subjects

Pregnant female Sprague–Dawley rats were purchased from Möllegaard Breeding Centre, Denmark. The animals were housed under constant conditions of temperature (24°C), humidity (50% RH), and dark–light illumination (lights on 0600–1800) and had free access to food [SDS RM1.(E)] and water. The individual rats were kept in separate standard macrolone cages measuring 40 × 25 × 18 cm for at least 4 days before delivery. After delivery, a mother and her pups were kept together in the mother's home cage pending use in experiments. Two days after delivery, each litter was culled to 10 pups.

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Drugs

The following drugs were used: buspirone, diazepam, 8-OH-DPAT (Sigma), and chlordiazepoxide (Roche). The drugs were dissolved in 0.9% saline and injected SC in the neck region 30 min before testing. The injected volume was 10 ml/kg body weight.

Procedure

Before drug treatment, a mother was removed from a home cage and her litter was covered with a pad of cotton. Five minutes after the removal of the mother five pups were treated with a drug or saline, and, after an additional 10 min, the remaining five pups were treated. All pups in the litter were kept together in their home cage between injection and testing. The individual pups in a five-pup group were respectively treated with vehicle (saline) and four concentrations of the drug. The two, five-pup groups of a litter were given the same treatment and each drug was given to a total of 10 five-pup groups (i.e., five litters). Thirty minutes after drug administration, ultrasound vocalization (USV) was monitored (for 5 min) individually for each pup in a five-pup group. USV was registered by using equipment consisting of five identical plastic cylinders with temperature-controlled (19.0±0.1°C) aluminum floors and crystal microphones mounted at the top. The sensitivity of the microphones was −67 dB/V/μBar, with an optimum at 40 kHz, and the detection limit of the USV length was 0.25 ms. The signal was processed by two preamplifiers (CA3100), and the integration of the sinus signal was performed separately for the + and − waves. The USV-registering system was connected to counters displaying the cumulative duration of calling every second during the test period by means of an electronic gate and a stable 50-kHz generator.

Two minutes after USV registration, the rat pups were killed by cervical dislocation and trunk blood was collected in heparinized tubes. The blood samples were centrifuged at 1800 × *g* for 10 min, and the plasma was stored at −20°C until analysed.

Corticosterone Assay

Individual concentrations of plasma CORT were measured in duplicate by using antiserum from Radioassay System Laboratories (Carson, CA) and performing radioassay on unextracted plasma in which binding proteins had been denatured by dilution with a pepsin solution (1,25). The intra-assay variance was below 5%.

Statistical Analysis

The data were analysed by Friedman two-way analysis of variance (ANOVA) with multiple comparisons. Significance levels were adjusted for multiple comparisons (Bonferroni corrections). Nonparametric trend analysis was performed by using the Page test (24).

RESULTS

The benzodiazepines diazepam and chlordiazepoxide dose-dependently ($p < 0.001$) decreased USV in isolated rat pups. Neither of these drugs affected the plasma corticosterone concentration, although a nonsignificant increase in the concentration was observed at the highest dose of chlodiazepoxide (Fig. 1). The nonbenzodiazepine drugs buspirone and 8-OH-DPAT caused a dose-dependent decrease ($p < 0.001$) in USV. In fact, at the highest dose the USV was nearly eradicated, which is in sharp contrast to the effects of the benzodiazepines, which even at the highest sedating dose did not completely inhibit the USV (Fig. 1). There was also a dose-dependent increase ($p < 0.001$) in plasma corticosterone concentration following treatment with buspirone or 8-OH-DPAT. Nonparametric trend analysis of the corticosterone data showed highly significant trends ($p < 0.001$) for buspirone and 8-OH-DPAT whereas no significant ($p > 0.05$) trend was found for diazepam and chlordiazepoxide. It was also found that the trend for the nonbenzodiazepines collectively differed ($p = 0.014$) from that of the benzodiazepines.

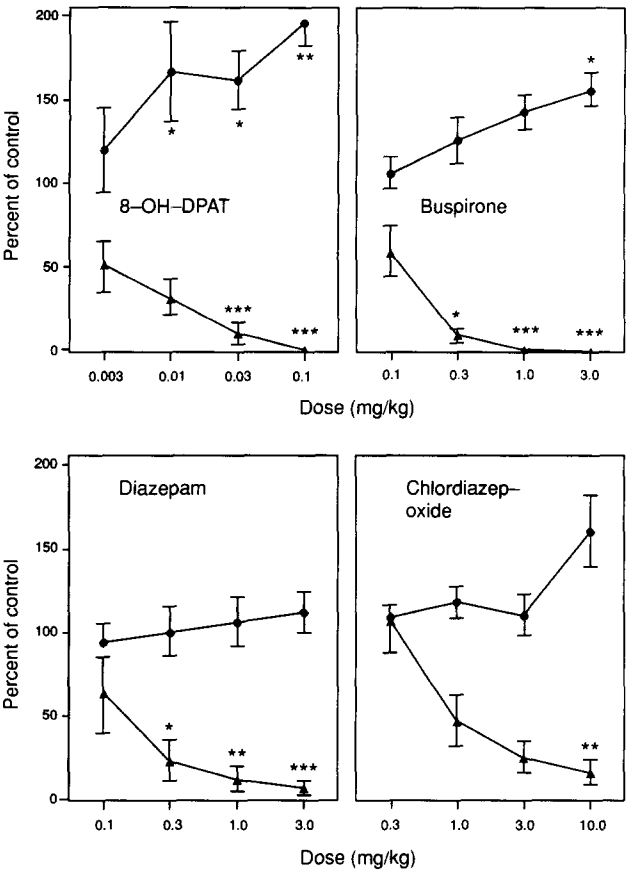

FIG. 1. Effects of various doses of 8-OH-DPAT, buspirone, diazepam, and chlordiazepoxide on ultrasound vocalization (▲) and plasma corticosterone concentration (●) in isolated 10-day-old rat pups immediately after sound recording. Ten animals were used at each dose. Statistically significant differences from the vehicle-treated controls at $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ probability levels, respectively.

DISCUSSION

During the first 2 weeks of postnatal development, rat pups respond only weakly to environmental stressors known to elicit a dramatic increase in plasma concentrations of CORT in adult animals (23,27). However, during this stress-nonresponsive period, environmental manipulations induce USV in rat pups, a behaviour that can be attenuated by administration of anxiolytic drugs (9).

Consistent with earlier findings (11,20), the present study

showed that administration of the 5-HT_{1A} agonists buspirone and 8-OH-DPAT dose-dependently suppressed rat pup ultrasonic vocalization. In addition, it was found that these 5-HT_{1A} agonists activated the pituitary-adrenocortical axis in 10-day-old rat pups. Thus, the discrepancy between the anxiolytic-like effect and the neuroendocrine stress response seen by other workers (3,16,26) in adult animals after treatment with 5-HT_{1A} agonists was also found in the present experiments in 10-day-old rat pups. The benzodiazepine diazepam exerts an anxiolytic-like effect in both adult rats and isolated rat pups when administered at a dose range that does not alter basal plasma CORT levels [(19) and the present study]. Similarly, chlordiazepoxide significantly attenuated USV without affecting the plasma CORT level. Only at a high, sedating dose (10 mg/kg) of chlordiazepoxide was there a nonsignificant increase in plasma corticosterone level. Earlier studies (8) have shown that the number of USVs was decreased by doses of diazepam and chlodiazepoxide that did not affect the locomotor activity. This effect was probably mediated via the benzidiazepine receptor, as indicated by the fact that pretreatment with the benzodiazepine receptor antagonist Ro 15-1788 completely abolished the effect of diazepam (14).

There is substantial evidence suggesting that stimulation of presynaptic 5-HT_{1A} receptors, which decreases the activity of the raphe neurons, is correlated not only with an anxiolytic-like effect (4) but also with the inhibition of USV in rat pups. To explain the paradoxical effects of buspirone and 8-OH-DPAT (i.e., their anxiolytic-like effect on USV and the concomitant stimulatory effect on the pituitary-adrenocortical axis) it may be assumed that these drugs act as a full agonist at somatodendritic 5-HT_{1A} receptors but also stimulate postsynaptic receptors mediating the release of corticosterone-releasing factor (17).

As mentioned above, ultrasonic calling by rat pups in conjunction with isolation has been described as distress vocalization, and the pharmacological attenuation of this USV has been suggested to be a useful tool in selecting new drugs with potential anxiolytic efficacy (28). However, whether this behaviour is elicited by social separation per se or is a physiological reaction to environmental changes remains to be elucidated. Exposure to environmental stressors does not seem to activate the pituitary-adrenocortical axis in rat pups that are less than 14 days old (23). The hypothalamic content of CRF increases gradually from birth. Hence, it may be assumed that the pharmacological activation of postsynaptic 5-HT_{1A} receptors, but not the benzodiazepine receptor, triggers the release of CORT in the 10-day-old rat pup, and that exposure to environmental stressors does not elicit a release of corticosterone in rat pups of that age.

In conclusion, the present results indicate that when measuring stress response as an increase in the concentration of plasma coricosterone, response interpretations may differ markedly according to whether the activation of the pituitary-adrenocortical axis is elicited by environmental stressors or by pharmacological manipulations.

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