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Neuroendocrine and Behavioral Responses During Conditioned Active and Passive Behavior in the Defensive Burying/Probe Avoidance Paradigm: Effects of Ipsapirone

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KORTE, S. M., G. A. H. BOUWS, J. M. KOOLHAAS AND B. BOHUS. Neuroendocrine and behavioral responses during conditioned active and passive behavior in the defensive burying/probe avoidance paradigm: Effects of ipsapirone. PHYSIOL BEHAV 52(2) 355-361, 1992.—Plasma epinephrine (E), norepinephrine (NE), and corticosterone (CORT) concentrations were determined in the rat before, during, and after a 15-min exposure to a nonelectrified probe one day after receiving electric shock (1.5 mA) through a probe mounted on the wall of the home cage. Rats displayed burying (active coping) if sawdust was provided on the floor and immobility (passive coping) if bedding was absent both during training and testing. The conditioned burying was accompanied by high plasma NE but low E and CORT concentrations, whereas immobility was associated with high CORT and low NE levels. A forced switch from the active to passive coping (training with and testing without sawdust) led to the highest rise in E concentration. The 5-HT1A agonist ipsapirone, with anxiolytic properties, dose-dependently (0.5 and 2.5 mg/kg, IV) reduced defensive burying behavior and increased the amount of time spent on feeding behavior in the presence of bedding material. Both plasma E and CORT levels were further elevated by the higher dose of ipsapirone. In the absence of bedding material, ipsapirone failed to affect immobility behavior, but it dose-dependently elevated the stress-induced increase in E, NE, and CORT concentrations. Accordingly, the behavioral anxiolytic action of the 5-HT1A agonist ipsapirone was restricted to active coping, whereas neuroendocrine activation by the drug was present in all conditions. It is suggested that the effects of ipsapirone on behavioral coping and neuroendocrine regulation are produced by different populations of 5-HT1A receptors in the brain.

Norepinephrine  Epinephrine  Corticosterone  Ipsapirone  5-HT1A receptor  Coping  Stress

The suggestion that serotonin (5-HT) may play a major role in anxiety has a long history (61). The recent development of novel compounds with high selectivity for subtypes of serotonergic receptors has lead to a reinvestigation of the link between 5-HT, stress, and anxiety. 5-HT agonists, particularly those with high affinity for the 5-HT1A receptor such as ipsapirone, are commonly used as anxiolytics (12-14,20,41,42). In the course of studying brain mechanisms of anxiety and stress, ipsapirone can be used as a tool to investigate the 5-HT1A receptor involvement. The anxiolytic effect of ipsapirone is mediated by suppression of serotonergic neuronal activity via a full agonistic action on presynaptic 5-HT1A autoreceptors located in the dorsal raphe nucleus (12,13,17,22,24,25,49,53,54) and/or via an inhibitory action at postsynaptic 5-HT1A sites in the septo-hippocampal system (2,28,40,50).

A novel aspect of anxiolytics is their potential use as antistress drugs. However, data concerning the effects of 5-HT1A agonists on stress responses are conflicting (15,52,34,35,37). Based on previous studies in our laboratory, we hypothesize that these seemingly contradictory results might be due to the effects of the 5-HT agonist under different experimental stress conditions or to the different measures of stress responses. For example, ipsapirone has antistress and anxiolytic-like effects on heart rate changes and behavioral responses in animal models of anxiety (35,37). However, if plasma levels of circulating catecholamines and adrenal corticosterone were determined as classical measures of neuroendocrine stress responses, ipsapirone further elevated these responses after inescapable stress (34). An important issue in stress research is to what extent the environment allows active or passive coping. It is well known that these different behavioral styles of coping are associated with different physiological and neuroendocrine response patterns (3,11,15,23,33). Therefore, in the present study the effects of the 5-HT1A agonist ipsapirone were investigated in the defensive-burying paradigm (56). This

1 Requests for reprints should be addressed to S. M. Korte.
procedure allows both active and passive coping. The present study addresses the question as to whether serotonergic mechanisms are differentially involved in the behavioral and neuroendocrine responses during both active and passive coping, as well as after a forced shift from active to passive coping.

METHOD

Animals

Male Wistar rats weighing 290–340 g at the beginning of the experiments were used. One week before the experiment the rats (six in a cage) were separated and housed individually in clear Plexiglas cages (25 × 25 × 30 cm) on a 12 h light–dark regime (lights on between 0730 h–1930 h) at room temperature of 21 ± 2°C. All animals had free access to standard food (Hope Farms rat chow) and water. The experiments were carried out between 0900 and 1300.

Surgery

A silicon heart catheter (0.95 mm o.d., 0.50 mm i.d.) was inserted through the right jugular vein (51) of the animal, under complete ether anesthesia. This method allows frequent blood sampling in undisturbed, freely moving rats (59). After each sample the same quantity of heparinized donor blood was given to avoid diminution of the blood volume with related changes in hemodynamics (51). Donor blood was obtained from unstressed rats with permanent heart catheters. The rats were given 1–2 weeks to acclimatize to separation and recover from surgery.

Drug Treatment

Ipsapirone (TVX 7821, Troponwerke GmbH and Co., Cologne, Germany) was dissolved in saline. Injections were given intravenously (IV) in a constant volume of 1.0 ml/kg. Two doses of the drug (0.5 and 2.5 mg/kg) were used. This dose range has shown to possess anxiolytic-like activity in a pilot study. Each rat received only one dose of the drug 10 min before the presentation of the stressor.

Apparatus

The animals were tested in their own home cage. The floor was either covered with wood shavings or the bedding material was removed 2 h before the start of the experiment. A removable teflon probe (6.5 cm long, 1 cm in diameter) was placed 6 cm above the floor, inserted through a small hole in the center of the front wall of the Plexiglas cage. Two exposed wires (0.5 mm in diameter) were each wrapped (25 times) independently around the probe. Whenever the animal touched both wires simultaneously with some part of its body an electric current (1.5 mA) was delivered to the animal. At the same time, two cumulative counters were triggered; one to register the number of contacts with the probe and the other to monitor the duration of contacts.

Procedure

The paradigm is based on an electrified probe mounted on one wall of the animals’ home cage. Rats tend to explore this probe, but after shock experience they react actively with pushing and spraying the bedding material toward and over the electrified probe (i.e., burying) (11,43,55–57). However, in an environment without bedding material, where the active burying option is not possible, the rats adopt a passive strategy, i.e., immobility in locations away from the probe (8). In the present study the rats were tested 24 h after the shock. This means that the procedure investigated the conditioned consequence of former punishment rather than the direct effect of shock.

More precisely, on day 1 the shock probe was inserted in the home cage for 15 min, either in the presence or in the absence of wood shavings. Upon touching the probe, the rat received an electric shock (1.5 mA). The shock circuit was left on during the entire period [repeated shock-probe procedure as described by Treti and Fundytus (57)]. All rats received similar amounts of electric shock. This phase is specified as acquisition (training) period. On day 2 vehicle or the drug was injected (IV) 10 min before presentation of the nonelectrified probe in the home cages of the rats. This phase is referred as retention (test) period. Two doses of the drug (0.5 and 2.5 mg/kg, IV) and saline were used. Every drug or vehicle-treated group consisted of six animals. Accordingly, three groups of 18 animals were trained and tested under the following conditions:

1. presence of bedding material during both acquisition and the retention session (+/+);
2. bedding material available during acquisition but not at the retention (-/+);
3. no bedding material available either on day 1 or on day 2 (-/-).

In a pilot study the rats did not perform a rapid shift from the passive to the active strategy. Therefore, the group (-/+1) was not included in these studies.

Blood Sampling

The experiments were performed in the home cages of the rats. Two hours before the start of the experiment the animals were connected with a polyethylene blood sampling tube (0.4 mm length, 1.45 mm o.d. and 0.75 mm i.d.) in their home cages. Blood samples were taken before (at t = -10 and t = - 1 min) and during presentation of the nonelectrified probe (at t = 1.5, 10, and 15 min). Blood samples taken during behavioral measurements (at t = 5 and 10 min) are presented as 1 mean value.

Behavioral Measurements

The behavior of the rats during the presentation of the nonelectrified probe on the test day (between t = 5–10 min) was recorded on videotape. This behavior was classified into 4 categories:

1. defensive burying—moving toward the shock probe and spraying or pushing the bedding material toward the probe with rapid movements of the snout or forepaws (43);
2. immobility—the animal is completely motionless and the body weight is supported by its limbs;
3. rearing—standing on the hind legs only; forepaws either free or leaning against the wall of the cage;
4. eating—chewing chow or feces.

Time spent on the display of each type of behavior was recorded. The observer was always the same person and was not familiar with the drug-treatment schedule.

Chemical Determinations

Blood samples of 0.45 ml were withdrawn for determination of plasma epinephrine (E), norepinephrine (NE), and corticosterone (CORT). The samples were immediately transferred to chilled (0°C) centrifuge tubes containing 0.01% EDTA as antioxidant and 10 μl heparin solution (500 IU/ml) as anticoagulant. Blood was centrifuged at 4°C for 10 min at 5000 rpm, and 100 μl of the supernatant were stored at -20°C for CORT
and at -80°C for the catecholamine measurements. Plasma CORT was measured by means of reversed phase high performance liquid chromatography HPLC (7). Determination of plasma catecholamine concentrations was performed by HPLC in combination with electrochemical detection (44,48).

Statistics

Behavioral data were analyzed using the Kruskal-Wallis ANOVA followed by the Mann-Whitney U-test. The neuroendocrine data were evaluated by means of an one-way analysis of variance (ANOVA) of STATS. Further analyses were made by post hoc t-tests to determine the source of the detected significances in the ANOVAs. A probability level of p < 0.05 was taken as significant.

RESULTS

Conditioned Behavioral Response in Defensive Burying/Probe Avoidance (CDB) Paradigm

Figure 1 shows the behavioral response of the saline-treated animals to the presentation of the nonelectrified probe on the test day. As expected, the animals who had bedding material available both during the training and test sessions (+/+) spent most of their time on defensive burying. Animals which had no wood shavings in their home cages during test conditions (+/-) and (-/-) displayed mostly immobility independent of the training conditions.

The Effects of Ipsapirone on the Behavioral Response

Figure 1 also shows that ipsapirone-treated rats with wood shavings available both on day 1 and day 2 (+/+) displayed less defensive burying in a dose-dependent way than did vehicle-treated controls (p = 0.054; Kruskal-Wallis test). The higher dose (2.5 mg/kg, IV) of the drug resulted in a significant effect compared to controls. Meanwhile, the time spent on eating behavior was increased (p = 0.015; Kruskal-Wallis test). This was significant for both the lower and the higher dose of the drug (p < 0.01 and p < 0.05, respectively). The duration of rearing behavior was diminished in a dose-dependent way (p = 0.006; Kruskal-Wallis test). The higher dose of the drug had a significant effect (p < 0.01). Ipsapirone failed to affect the duration of immobility behavior if bedding material was not available during the test (+/-) and (-/-)). In all conditions ipsapirone-treated animals displayed less rearing behavior relative to vehicle-treated controls (p = 0.004; Kruskal-Wallis test). The effect of the higher dose was significant (at least p < 0.05).

The Neuroendocrine Response

Figure 2 shows the patterns of neuroendocrine responses in animals under different testing conditions. Rats which had no bedding material available on the test day (+/-) and (-/-)) showed higher plasma CORT and lower NE than those animals which did have bedding material (+/+).

The ANOVAs of CORT levels revealed significant effects of the factor condition at T = 5 min, F(2, 14) = 7.16, p = 0.007, at t = 10 min, F(2, 14) = 8.40, p = 0.004, and at t = 15 min, F(2, 14) = 8.22, p = 0.005, respectively. The magnitude of the CORT responses of the three animal groups were as follows: (+/-) > (-/-) > (+/+) as determined by significantly higher levels of CORT in the (+/-) and (-/-) groups relative to (+/+) at t = 5, 10, 15 min (at least p < 0.05). The ANOVAs of plasma NE levels in animals under different testing conditions indicated significant differences at t = 5 min, F(2, 15) = 4.61, p = 0.027.

The ANOVAs of plasma NE showed no significant differences. There was no significant difference between the received amounts of electric shock [group (+/+)]: 1.3 ± 0.2 contacts and 16.4 ± 2.5 ms duration: group (+/-): 1.5 ± 0.2 contacts and 10.9 ± 3.0 ms duration: group (-/-): 1.4 ± 0.2 contacts and 13.2 ± 2.7 ms duration].

The Effects of Ipsapirone on the Neuroendocrine Response

Figure 3 shows the effect of ipsapirone on levels of CORT, NE, and E in animals which were tested under different conditions. Although blood samples were taken at the start (t = 5 min) and the end (t = 10 min) of the behavioral measurements, the two data did not significantly differ and, therefore, were pooled for the purpose of data reduction. Comparison of the ipsapirone-treated animals relative to the vehicle-treated controls under the same testing conditions showed that plasma CORT and E levels were elevated in the drug-treated animals. Ipsapirone (2.5 mg/kg) caused significant elevations of plasma CORT (p < 0.001 for each group) and E levels in all conditions [(+/+), p = 0.002: (+/-) and (-/-), p < 0.001]. Plasma NE levels were not affected if the rats were trained with bedding material while a significant elevation was found under (-/-) condition (p = 0.002). The analysis of the data across test conditions failed to show significant differences for the lower dose of ipsapirone. For the higher dose (2.5 mg/kg, IV) differences were found for plasma E levels, F(2, 14) = 5.47, p = 0.017. The (-/-) group showed higher levels of E relative to the (+/) and (+/-) groups (at least p < 0.05).
FIG. 2. Plasma epinephrine, norepinephrine, and corticosterone levels in rats before and during 15-min exposure to a nonelectrified probe in their home cages one day after the shock procedure (day 2) under different testing conditions: day 1 and day 2 bedding material available (+/+); day 1 bedding material available, but not on day 2 (+/-); neither day 1 nor day 2 bedding material available (-/-). Data are expressed as mean ± SEM for five to six rats per condition. *p < 0.05; **p < 0.01.

FIG. 3. The effect of ipsapirone (0.5 and 2.5 mg/kg, IV) on plasma epinephrine, norepinephrine, and corticosterone in rats during exposure to the nonelectrified probe in the test session. The animals were exposed to the probe in the presence (+) or absence (-) of bedding material during the shock session (day 1) and the test session (day 2). Data are expressed as mean ± SEM for five to six rats per condition and represent the mean of the samples taken at the beginning (T = 5 min) and end (T = 10) of the behavioral observation period. *p < 0.05; **p < 0.01.

DISCUSSION

The present study reinforces the view that various behavioral styles of coping are associated with different patterns of peripheral sympathetic, sympathoadrenomedullary, and pituitary-adrenocortical activation. The specific response is determined by learned coping strategies and the predictability of the test condition. Treatment with the 5-HT1A agonist, ipsapirone, led to activation of the adrenomedullary and the adrenocortical system under all conditions. In contrast, a behavioral anxiolytic effect of ipsapirone was observed only in the active coping situation. This suggests a dissociation of the organization of serotonergic mechanisms involved in behavioral and neuroendocrine stress responses.

In the present study, the neuroendocrine consequences of conditioned fear were investigated in animals that use either an active (defensive burying) or a passive strategy (immobility) to cope with a conditioned aversive stimulus. During active coping
(+/-), defensive burying was accompanied by high plasma NE but low E and CORT levels, suggesting a selective peripheral sympathetic activation. This sympathetic activation is probably secondary to general activity, involving skeletal muscle exertion (9,47). In the passive coping experiment (-/-), the animals expressed immobility behavior, accompanied by pituitary-adrenocortical activation resulting in high plasma levels of CORT. These data support the hypothesis that passive coping leads to activation of the adrenal cortex. The elevation of plasma E concentrations, as reported to occur during acute threat (10), is practically absent in a conditioned stress situation.

There is a large body of evidence that serotonin (5-HT) is involved in anxiety (4,14,29,35,37,52,61). The 5-HT<sub>1A</sub> agonist, ipsapirone, shares activity with the classical benzodiazepine anxiolytic diazepam in a number of animal models of anxiety (13,53), but does not directly affect GABAergic neurotransmission. In the present study, ipsapirone had an anxiolytic effect in the active coping animals, since it led to a dose-dependent reduction of the time spent on defensive burying. There was no change in total activity since the drug-treated animals spent substantial amount of time on eating. Recently, it was reported that the 5-HT<sub>1A</sub> receptor agonists stimulate food intake in freely moving rats (16). This probably occurs by activation of autoreceptors on the cell bodies of 5-HT neurons which is followed by a decrease in 5-HT release at the terminals in the hypothalamic paraventricular nucleus (PVN) (16,18,26,27,30). The increase in food intake raises the question whether the drug reduced anxiety, or rather caused a shift in the responses to the stressful situation (e.g., binge eating). Ipsapirone failed to have an anxiolytic effect in animals displaying passive behavior (immobility). This is in contrast with our earlier findings in which ipsapirone diminished a generalized/anticipatory bradycardiac stress response one day after the experience of absence of control during electric footshock (37). Taken together these results suggest that 5-HT<sub>1A</sub> receptor mechanisms are only partly or differentially involved in the behavioral and cardiac stress responses in the passive coping situation.

Ipsapirone treatment led to an exaggerated activation of both the adrenomedullary system and the hypothalamic-pituitary-adrenocortical (HPA) axis in all test situations. This is in agreement with other studies showing that ipsapirone and related compounds (e.g., gepirone, buspirone, 8-OH-DPAT) may act as an agonist on post-synaptic 5-HT<sub>1A</sub> receptors probably located in the hypothalamic paraventricular nucleus (PVN), resulting in a dose-related increase in plasma CORT and E (21,36). This indicates that a specific adrenomedullary and adrenocortical activation occurs (1,5,6,9,23,32,36). Neuronal NE outflow is less diminished a generalized/anticipatory bradycardiac stress response one day after the experience of absence of control during electric footshock (37). Taken together these results suggest that 5-HT<sub>1A</sub> receptor mechanisms are only partly or differentially involved in the behavioral and cardiac stress responses in the passive coping situation.

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REFERENCES


