Pharmacology Biochemistry and Behavior, Vol. 64, No. 3, pp. 473–477, 1999

0091-3057/99/$–see front matter

PII S0091-3057(99)00097-0

Preexposure to Drug Administration Context Blocks the Development of Tolerance to Sedative Effects of Diazepam

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Received 27 March 1998; Revised 5 January 1999; Accepted 15 January 1999

MARIN, R. H., M. F. PEREZ, D. DUERO AND O. A. RAMIREZ. *Preexposure to drug administration context blocks the development of tolerance to sedative effects of diazepam.* PHARMACOL BIOCHEM BEHAV **64**(3) 473–477, 1999.—The development of tolerance to benzodiazepine (BDZ) and other psychoactive agents such as morphine, alcohol, and barbiturates is thought to be a contingent or learning phenomenon. In a previous report, we demonstrated a positive correlation between the development of tolerance to the sedative effects of diazepam (DZ) and hippocampal synaptic plasticity. The results of the present work show that the development of tolerance to the hypolocomotor action of DZ (5 mg/kg) for 4 days and the associated increase in synaptic plasticity are context specific. Because animal preexposure to the drug administration context blocks both the tolerance sedative effects of DZ and the increased hippocampal synaptic plasticity, observed after 4 days of DZ administration, we propose the increased synaptic plasticity on hippocampal development as one of the biological substrates to the tolerance to DZ. Besides, the continuous administration of DZ did not induce a conditioning opponent response in these animals. © 1999 Elsevier Science Inc.

Diazepam Tolerance Synaptic plasticity Hippocampus Open field Rats

CHRONIC administration of benzodiazepine induces rapid tolerance development to the sedative effect, motor disturbances, and motor relaxation evidenced by these drugs (3,9,10,23). Recently, several authors have reported that the administration of glutamate antagonists prevents the development of tolerance to the sedative effects of diazepam (DZ) in rats and mice (11,16,26,27).

Long-term potentiation (LTP) of synaptic transmission is a relevant phenomenon seemingly linked to neural information storage (29). In the hippocampal formation, LTP can be produced by repetitive activation of afferent pathways (6,17). It is believed that glutamatergic receptors, such as *N*-methyl-D-aspartate (NMDA), participate in the induction of LTP (13). Phosphonovaleric acid derivative (APV), an antagonist to NMDA receptors, blocks the induction of LTP and the acquisition of different behavioral responses (21). A contingence learned phenomenon underlying the development of tolerance to several psychoactive drugs has been suggested (5,15,31,33). The NMDA is a kind of receptor whose blockade impairs the development of rapid tolerance to DZ, these receptors have been related to neural and behavioral plasticity (1,2,4,5,30). Moreover, NMDA receptors have also been related to opiate tolerance and dependence (32). More recently, our laboratory has reported an increased hippocampal synaptic plasticity during the development of rapid tolerance to the hypolocomotor effects of DZ (19). Considering this finding and the role of the hippocampal LTP as a paradigm of learning and memory, we decided to investigate whether the increased hippocampal synaptic plasticity observed during the development of rapid tolerance to DZ might be a biological substrate, supporting the contingency of this phenomenon. The second aim of this study was to examine if repeated administration of

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DZ results in conditioned behavioral responses that can partly account for the development and expression of functional tolerance to this drug.

EXPERIMENT 1

Because tolerance to different psychoactive drugs exhibits many basic features of classical conditioned phenomena (27), in the present study we investigate whether rapid tolerance development to the hypolocomotor effect of diazepam is context specific. Animals were either pre- or nonexposed to the context of the drug administration (conditioned-inhibition paradigm) before examining the effect of DZ (5 mg/kg/day) for 4 days on the locomotor activity in an open-field test.

Methods

Animals. In all, 46 male Wistar rats 60–75 days old and weighing 200–260 g were used. Animals were housed in groups of six in their home boxes and kept under a 12 L:12D cycle (light on at 0700 h) and regular temperature conditions (22 ± 1°C). Food and water were available ad lib.

Open-field apparatus. The spontaneous locomotor activity was evaluated in a square-wooden open field. Its sides were measured 60 cm in length by 60 cm in height. The lit gray surface was divided into 16 squares, each 15 cm wide, and illuminated by a 100-W overhead bulb. The open field was placed in a sound-proof room lighted by a 40-W bulb.

Procedure. Rats from the two experimental groups were carried in their home boxes to the experimental sound-proof room, where they were preexposed to the drug treatment administration context (preexposed rats). Animals were individually taken by the experimenter, the "syringe" (without needle) with either DZ (7-Chloro-1-methyl-5-phenil-3H-1,4-benzodiacepin-2(1H)-one, Roche, 5 mg/kg) or vehicle (distilled water with a drop of Tween 80 and propilenglicol 5%) was pressed on the abdomen region without drug administration. Afterwards, animals were placed back in their home boxes for 30 min and returned to their home room. Two hours later, subjects were carried again to the same experimental room, the procedure was repeated, but they received either a DZ or vehicle injection. Thirty minutes after either schedule injection, each animal was individually placed in the open field for a 10-min period, during which locomotor activity was assessed by the number of squares entered. Later, animals were placed back in their home boxes and returned to their home room. This procedure was followed for 4 days. Twenty-four hours after the last training day animals were sedated with a 50:50 mixture of CO_2/O_2 and immediately sacrificed by cervical dislocation, for electrophysiological studies.

On the other hand, preexposure control groups (non preexposed rats) were carried in their home boxes to the experimental room, as preexposed groups, but they directly received either a DZ injection or vehicle injection. Thirty minutes after either injection, each animal was individually placed in the open field for a 10-min period, during which locomotor activity was determined as described for the preexposed rats. Rats were sacrificed for electrophysiological studies 24 h after the last injection. All subjects used in this group were either rats showing DZ tolerance developed throughout the 4 days training and rats that received vehicle injection during the same period. These conditions meet the standards for care of laboratory animals as outlined in the NIH Guide for the Care and Use of Laboratory Animals (1996).

Electrophysiological experiments. The subjects were the preexposed or nonpreexposed rats administered with DZ or vehicle for 4 days. The hippocampal slices used in electrophysiological experiments were obtained from animals sacrificed 24 h after the last injection.

Electrophysiological experiments were done using the in vitro hippocampal slice preparation described elsewhere by Ramirez et al. (22). Briefly, experimental and control rats 60–75 days old were sacrificed between 1100 and 1200 h to prevent variations caused by circadian rhythms or nonspecific stressors (28). The hippocampal formation was dissected and transverse slices, approximately 400 μm thick, were obtained and placed in a recording chamber, perfused with standard solution saturated with 95% O_2 and 5% CO_2. Rate of perfusion was 2–3 ml/min, and the bathing solution temperature was kept at 28°C. A stimulating electrode was placed in the perforant path (PP), and a recording microelectrode was inserted in the dentate granule cell body layer. Only slices showing a healthy response were included in this electrophysiological study. Ten field potentials that responded to the stimuli were sampled at 0.2 Hz, averaged on line using a PC computer, and the data thus obtained were stored in diskettes for further analysis. Once no changes were observed in the amplitude of the response, which included population spike (PS), for 20 min, the intensity of the electrical stimulus to the PP was set at the value that would elicit spikes approximately 30% of the maximum response. The long-term potentiation (LTP)-eliciting frequency threshold was determined as described by Ramirez et al. (22). Tetanus consisting of a train of pulses (0.5 ms) of 1 s duration and with increasing frequency was delivered to the slice at intervals that ranged from 20 min up to 45 min, starting with a 5 Hz tetanus, whose intensity increased with each train to 10, 25, 50, 75, 100, 150 up to 200 Hz. Fifteen to 20 min after a tetanus, a new averaged response was recorded; when LTP was not observed, another tetanus at the next higher frequency was applied. LTP was considered to have occurred when the amplitude of the evoked population spike (Fig. 2) recorded after the tetanus had risen by at least 30% and remained from 20 min to 1 h. Once LTP was achieved, no further tetani were given.

Results

Locomotor activity. A repeated-measure two-way ANOVA on the number of squares crossed revealed a significant interaction, $F(3, 135) = 3.1$, $p = 0.028$ (Fig. 1) between the effects of preexposure (preexposed and nonpreexposed), drug administration (DZ and vehicle) and days of treatment (1,2,3, and 4). Newman–Keuls pairwise comparisons of means showed that locomotor activity on the first day was significantly lower in DZ treated rats (preexposed or not) (12.67 and 14.1 squares, respectively) than in their respective vehicle controls (35.86 and 48.88 squares, respectively) ($p < 0.01$). On the second day the locomotor activity was also significantly lower in DZ treated rats (preexposed or not) (9.26 and 27.6 squares, respectively) than in their respective vehicle controls (36.60 and 55.55 squares, respectively) ($p < 0.01$). From the third day of treatment, the locomotor activity of the nonpreexposed rats showed no significant differences between DZ and vehicle treated rats, but within the preexposed groups significant differences between DZ and vehicle treated rats ($p < 0.01$) were noticed even on the fourth day of treatment (Fig. 1). Moreover, the Newman–Keuls test also showed significant differences between DZ preexposed rats on the third (13.73 squares) and fourth (7.53 squares) days compared with DZ nonpreexposed rats on the same days (40.00 and 46.10 squares, respectively).

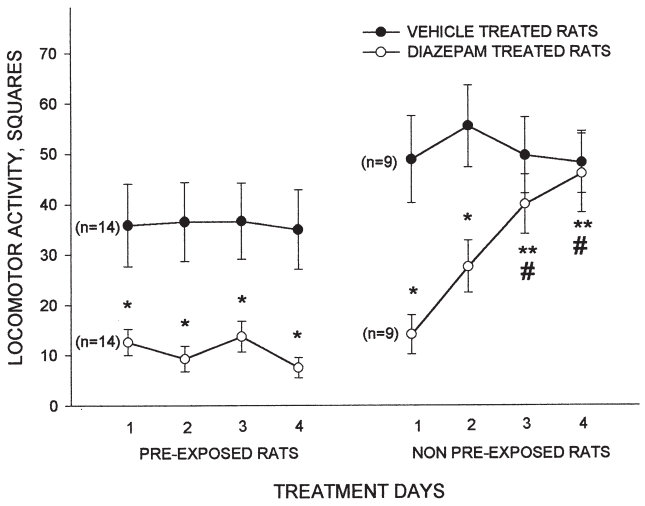

FIG. 1. Time course of changes in locomotor activity of preexposed and nonpreexposed rats to the context of drug administration with vehicle or diazepam treatment. Circles represent mean, and vertical bars the SEM. The number of animals is indicated in parentheses. *$p <$ 0.01 compared with their respective vehicle rats on the same day. **$p <$ 0.01 compared with DZ nonpreexposure rats on day 1. #$p < 0.01$ compared with DZ preexposed rats on the third and fourth day.

No differences in the locomotor activity were observed during the 4 days of treatment either within the vehicle control groups (preexposed or not) or within the DZ preexposed group (Newman–Keuls test). Figure 1 shows that rats preexposed to the drug administration context failed to develop the rapid tolerance phenomenon to the hypolocomotor effect of diazepam.

HippocampaI synaptic plasticity. Figure 2 shows the characteristic evoked field response in the granule cell layer of the dentate gyrus after single-pulse stimulation in the perforant path. It consisted of a gradual positive-going field excitatory postsynaptic potential (EPSP) with a sharp negative-going PS superimposed on the rising phase of the EPSP. The EPSP reflects synaptic currents at perforant path–dentate granule cell synapses in stratum moleculare, whereas the PS reflects the synchronous action potential discharge of granule cell bodies in stratum granulosum. Figure 2 in the right panel shows the increased amplitude of PS after an effective tetanus.

Two-way ANOVA on the threshold frequency to elicit LTP revealed a significant interaction, $F(1, 22) = 7.88.$; $p = 0.01$ (Fig. 3) between the effects of preexposed (preexposed and nonpreexposed) and drug treatment (DZ and vehicle). Newman–Keuls pairwise comparisons of means test showed that no differences were observed in the threshold rate to elicit LTP between the DZ and vehicle preexposed groups, but the threshold rate to elicit LTP was significantly lower in DZ nonpreexposed rats (13.33 Hz) than in their respective vehicle nonpreexposed (75.00 Hz) and in DZ preexposed (78.57 Hz) groups ($p < 0.005$).

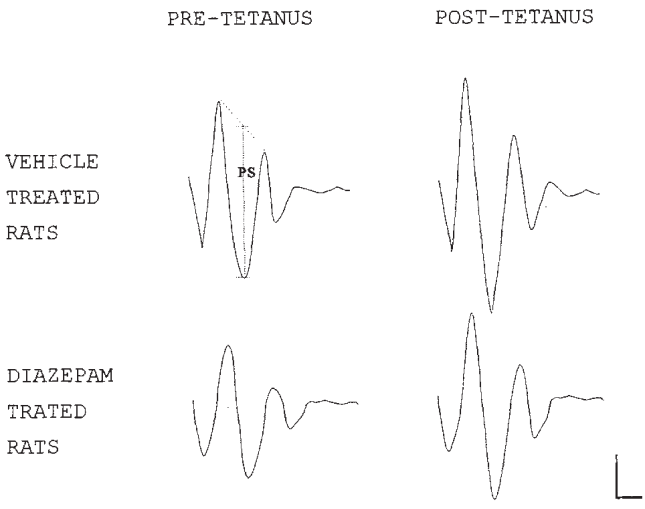

FIG. 2. Field potential of responses from granule cells layer of the dentate gyrus evoked by stimulation of the perforant path in a hippocampal slice. Pretetanus was delivered at 0.2 Hz; posttetanus shows the responses recorded after a train of high-frequency stimulation. Calibration bars represent: 5 ms and 0.25 mV. PS, population spike.

EXPERIMENT 2

Repeated administration of drugs often results in the conditioning of behavioral responses (24,25). These conditioned responses can be distinguished from other direct and indirect drug effects by the fact that under appropriate circumstances they can be elicited without administering the drug (8). To test this hypothesis in our experimental conditions we administered diazepam 5 mg/kg for 3 days, and on day 4, the animals received vehicle instead of drug.

Methods

Subjects. Altogether, 23 rats were used in this trial. The characteristics and conditions were the same as those in Experiment 1.

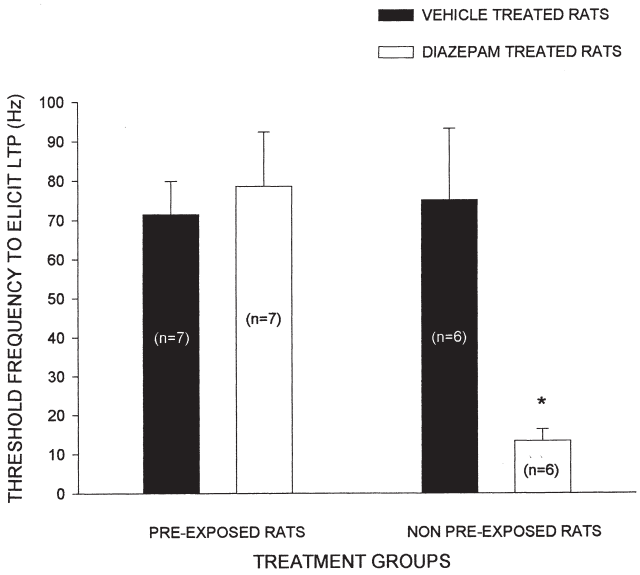

FIG. 3. Threshold frequency to elicit LTP in hippocampal slices from preexposed and nonpreexposed rats, treated with vehicle or diazepam (4 days). Bars represent the mean and vertical bars the SEM. The number of animals is indicated in parentheses. *$p < 0.01$ compared with respective vehicle nonpreexposed rats and compared with the preexposed groups.

Procedure. Two experimental groups were carried in their home boxes to the experimental room, where they received either a DZ or a vehicle injection. Thirty minutes after either injection, all animals were individually placed in the open field for a 10-min lapse, during which locomotor activity was assessed by the number of squares entered. Afterwards, animals were placed back in their home boxes and returned to their home rooms. This procedure was followed only 3 days, on day 4 all animals received vehicle injection.

Results

Figure 4 shows the time course of changes in locomotor activity of rats previously treated with vehicle and DZ (5 mg/kg/day) for 3 days and the effects of vehicle on day 4. A repeated-measure one-way ANOVA on the number of squares crossed revealed a significant interaction, $F(3, 63) = 3.18$, $p = 0.029$, between the effects of drug administration (DZ and vehicle) and days of treatment. Newman–Keuls pairwise comparisons of means test showed that the locomotor activity on the first day, was significantly lower in DZ treated rats (18.82 ± 3.19 squares) than in control animals (43.00 ± 7.82 squares) ($p < 0.01$). After the third day of treatment, the locomotor activity of DZ-treated and control rats did not show any significant differences. The ambulatory activity effect of the vehicle injection given on treatment day 4 did not show any differences either compared to their control group or to their activity on the day before (day 3, Fig. 4). Newman–Keuls test also showed significant differences between the DZ-treated group on day 3 (37.54 ± 6.84 squares) and the DZ-treated group on day 1 (18.82 ± 3.19 squares).

GENERAL DISCUSSION

Rats administered benzodiazepine for 4 days develop "rapid" tolerance to its hypolocomotor effect (8,9,19). Previous data from our laboratory also confirm an increased hippocampal synaptic plasticity after chronic DZ administration (19). In the present study we demonstrate that early previous exposure to the drug environment impairs both the tolerance to the hypolocomotor effect and to the increase in hippocampal synaptic plasticity reported after 4 days of (5 mg/kg) diazepam administration (Figs. 1 and 3). These results are in agreement with the learning hypothesis, put forward as a mechanism of the conditioned tolerance development to different psychoactive drugs (12). The long-term potentiation of synaptic transmission in the hippocampus has been extensively studied as a model of learning and memory. Besides, drugs blocking the LTP are also effective in impairing behavior in different tests (20,21). If the development of tolerance to DZ is a learning or contingent phenomenon, and the environmental clues are relevant, then our results concerning the impairment of the development of tolerance to the sedative effects of DZ, and the concomitant lack of increase in hippocampal synaptic plasticity, observed in animals after preexposure to the drug environment, may suggest the role of the hippocampal plasticity as a biological explanation for the contingent or learning interpretation of the tolerance to DZ. Supporting of this view is the fact that antagonists of NMDA receptors block the development of tolerance to the effects of cocaine on locomotor activity (5), to the analgesic effects of morphine (18,31), and to the motor-impairing effects of ethanol (15,32). In a recent review, Kalant (14) pointed out that it was impossible to know, when comparing tolerant with to nontolerant animals, whether a particular change in the brain of the tolerant subject was the mechanism responsible for the production of tolerance or merely a consequence of tolerance. Nevertheless, we can speculate that the change in the hippocampal synaptic plasticity is, at least, one of the biological mechanisms causing the development of tolerance to different neurological depressor drugs or just a consequence of the phenomenon underlying this learning process.

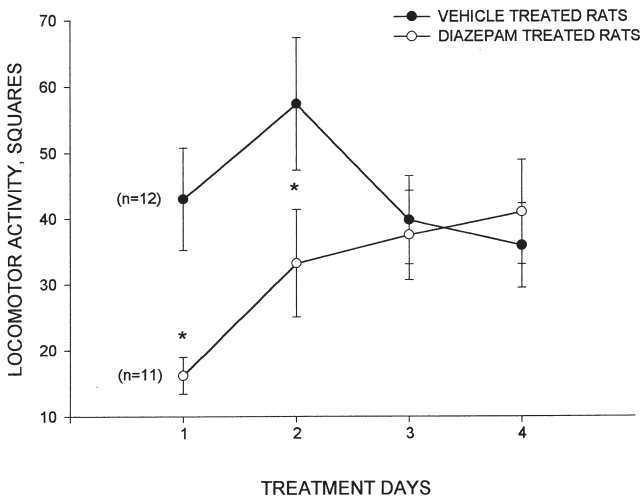

FIG. 4. Time course of changes in locomotor activity of rats treated with diazepam 5 mg/kg for 3 days, and on day 4 with vehicle, and their controls treated with vehicle along 4 days. Circles represent mean, and vertical bars SEM. The number of animals is indicated in parentheses. $*p < 0.01$ compared with their controls.

A conditioned drug-opponent response is thought to increase with drug exposure and add up with the unconditioned response to the drug resulting in tolerance (7). Looking into this hypothesis, in a different group of rats, we administered DZ for three days at the same doses; on day 4 we gave the subjects vehicle and evaluated their locomotor activity. Three days of treatment with DZ (5 mg/kg/day IP) led to a rapid loss of its depressant action on locomotor activity as evidenced by the number of squares entered. Unexpectedly, the vehicle injection given on day 4 of treatment did not show any differences in the open-field activity compared with either controls or with to the activity on the previous day (day 3) (Fig. 4). Two plausible hypothesis could explain the lack of compensatory response on day 4. One of them is the time during which the tolerance to DZ is developed. Apparently 3 days are possibly a short period of time to promote a conditioning of the behavioral response in these animals. Another possibility would be that, under our experimental conditions, we could not see a conditioned drug-opponent response because the locomotor activity observed was the maximum response possible.

Although some evidences have been accumulated to support the associative-tolerance hypothesis further, studies exploring other neurochemical systems and/or other areas of the brain should be made to elucidate the biological phenomenon underlying the psychoactive drugs tolerance development.

ACKNOWLEDGEMENTS

This research was supported by grants from CONICET and CONICOR (Argentina). The authors wish to thank Dr. Juan Carlos Molina for his useful criticisms.

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