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BRIEF COMMUNICATION

Blockade by Diazepam of Conditioned Fear-Induced Activation of Rat Mesoprefrontal Dopamine Neurons

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IDA, Y., A. TSUDA, K. SUEYOSHI, I. SHIRAO AND M. TANAKA. *Blockade by diazepam of conditioned fear-induced activation of rat mesoprefrontal dopamine neurons.* PHARMACOL BIOCHEM BEHAV **33**(2) 477–479, 1989.—The effect of diazepam on activation of the mesoprefrontal dopamine (DA) system by an emotional stress model without direct physical stimuli was examined. Environmental stimuli previously paired with inescapable footshock (conditioned fear) elicited increases in levels of the DA metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), in the medial prefrontal cortex and of plasma corticosterone in rats. The increases in both levels were blocked by pretreatment with diazepam (5 mg/kg, IP); such blocking effects were reversed by Ro 15-1788 (15 mg/kg, IP), the benzodiazepine (BZD) receptor antagonist. These data suggest that diazepam can block activation of mesoprefrontal DA neurons as well as hypothalamo-pituitary-adrenocortical system elicited by the conditioned fear paradigm. This action appears to be a specific action of BZDs mediated through BZD receptors. We suggest that blocking effects of BZDs on the hyperactivity of the mesoprefrontal DA neurons may be one neural mechanism of their anxiolytic actions.

Conditioned fear Mesoprefrontal dopamine neurons DOPAC Plasma corticosterone Diazepam Ro 15-1788

THE first report that 20 min of inescapable electric footshock stress selectively activates the mesoprefrontal dopamine (DA) system (13), suggested the hypothesis that this system plays an important role in the control of negative emotional states (anxiety and/or fear). The findings that the selective activation of the mesoprefrontal DA system by this stress model could be blocked by anxiolytic benzodiazepines (BZDs) such as diazepam (4, 7, 10) and these effects reversed by the BZD receptor antagonist, Ro 15-1788 (11), have supported the hypothesis and developed the pharmacological idea that the effect of BZDs on activity of the mesoprefrontal DA system may be one neural mechanism of their anxiolytic action which is mediated through BZD receptors. However, there remains the issue of whether or not inescapable electric footshock stress is a suitable stress model in which to test anxiolytic agents, because of the involvement of drug effects on various factors other than emotion, including motor activity, reactivity to electric shock and pain threshold. BZDs are known to possess not only anxiolytic actions but also sedative and muscle relaxant effects, and could influence nociceptive thresholds under stress (3,8). In order to test adequately the above hypothesis, the effects of BZDs should be tested in another emotional stress model where involvement of direct physical stimuli is excluded to the greatest degree possible.

It has been reported that conditioned fear, a nonphysical and emotional stress wherein rats are exposed to only environmental stimuli previously paired with inescapable footshock, elicited selective activation of mesoprefrontal DA neurons (1,5).

The aim of the present study was to determine whether activation of mesoprefrontal DA neurons induced by conditioned fear could be blocked by diazepam and these effects could be reversed by Ro 15-1788. The drug effects on plasma corticosterone levels (a peripheral index of stress responses) were also examined.

METHOD

Animals

Male Wistar rats, weighing 170–190 g, were used. Animals were housed in a temperature-controlled room (24 ± 1°C) with a 12-hr light-dark cycle, and were allowed free access to food and water.

Drugs

Diazepam (Nippon Roche, K.K,) and Ro 15-1788 (a gift from Nippon Roche, K.K.) were suspended in 0.3% carboxymethyl

cellulose sodium and were injected IP in a fixed volume of 0.3 ml/100 g weight.

Apparatus

A rectangular clear plastic box (93 cm in width, 99 cm in length and 53 cm in hight) was used for exposing rats to inescapable footshock. The electrified floor was constructed of stainless steel rods of 0.3 cm in diameter and spaced 1.3 cm apart (center to center). The apparatus was subdivided into 25 smaller compartments (18 × 19 × 53 cm) by clear plastic walls.

Experimental Procedure

Forty rats were randomly assigned to one of 5 groups of 8 rats each and were placed in the compartments of the plastic box for 40 min every day for 3 days for habituation to the novel environment. After 3 days of habituation, four groups of rats were exposed to 3.0-mA, 5-sec inescapable shocks on a fixed-interval of 30 sec for 30 min and subsequently left for 10 min to reinforce the conditioning to the environment. Control rats were placed into the compartments for the same duration of time but received no shock. After the shock session, all rats were returned to their home cages.

Twenty-four hours later, four shock groups of rats were treated with either Ro 15-1788 at 15 mg/kg or vehicle 10 min before and either diazepam at 2 or 5 mg/kg, or vehicle immediately before replacing into the respective compartments for 40 min to expose to the environmental stimuli previously associated with shock. Rats in the control group were injected twice with vehicle at the same time and placed into the compartments for 40 min.

Tissue Preparation and Biochemical Assay

Immediately after each experimental procedure, rats were decapitated and the brains were rapidly removed. The medial prefrontal cortex (MPFC) was dissected out after making a coronal slice (about 2 mm thickness) on the ice as previously described (1) and frozen on solid CO_2 immediately. Blood from the cervical wound was collected into heparinized tubes. Dissected brain tissues and separated plasma were stored at −80°C until assayed. Four days after the experiment, both levels of DA and its major metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), in the MPFC were determined by high performance liquid chromatography with electrochemical detection (10). Plasma corticosterone levels were determined fluorometrically by the method of van der Vies (14).

Statistical Analysis

All statistical comparisons were made by using Student's *t*-test (two-tailed).

RESULTS

As shown in Fig. 1, an exposure to the environmental stimuli for 40 min elicited significant increases in DOPAC levels in the MPFC (131% of control) and in plasma corticosterone levels (165% of control). Both increases in DOPAC levels in the MPFC and in plasma corticosterone levels were blocked in a dose-dependent manner by diazepam and the significant effects were obtained at a dose of 5 mg/kg. These effects of diazepam on the MPFC DOPAC and plasma corticosterone levels were significantly antagonized by pretreatment with Ro 15-1788 at 15 mg/kg. Any significant changes in DA levels in the MPFC were not observed between any experimental groups (data not shown).

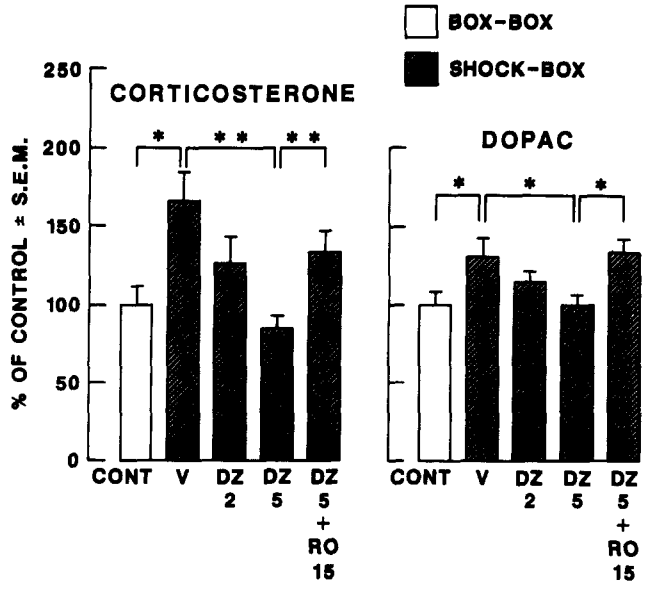

FIG. 1. Effect of diazepam (DZ) and/or Ro 15-1788 (RO) on levels of DOPAC in the medial prefrontal cortex and plasma corticosterone in rats exposed to environmental stimuli previously paired with inescapable electric footshock. Each value indicates the mean ± S.E.M. of 7–8 rats and are expressed as pecentage of the value obtained from box-box control (CONT) rats. Rats in five groups were treated IP with either diazepam at 2 or 5 mg/kg or vehicle (V) 10 min before and either Ro 15-1788 at 15 mg/kg or vehicle immediately before replacing into boxes for 40 min, respectively. Respective values of box-box control are: DOPAC in the MPFC 31.3 ± 2.1 ng/g; plasma corticosterone 23.9 ± 2.6 μg/dl. The horizontal bar indicates the statistical significance between the two groups compared by Student's *t*-test (two-tailed) (*$p<0.05$, **$p<0.01$).

DISCUSSION

In the present study, environmental stimuli previously paired with inescapable electric footshock elicited increases in DOPAC levels in the MPFC and in plasma corticosterone levels, a sensitive hormonal index of emotional responses by stress. These data are consistent with previous reports (1,5) reconfirming that the conditioned fear paradigm, an emotional stress model where the direct effects of physical stimuli were unlikely to be involved, produces activation of mesoprefrontal DA neurons as well as the hypothalamo-pituitary-adrenocortical system.

These increases in DOPAC levels in the MPFC and in plasma corticosterone levels were blocked by diazepam, and these effects of the drug were in turn antagonized by Ro 15-1788. These data indicate that diazepam has a blocking action on the activation of both the mesoprefrontal DA neurons and the hypothalamo-pituitary-adrenocortical system induced by conditioned fear, and that this action is mediated by BZD receptors. The present findings are in agreement with previous data showing that selective activation of the mesoprefrontal DA neurons by 20 min of inescapable electric footshock stress is blocked by diazepam (4, 7, 10), and that these effects are antagonised by Ro 15-1788 (11). Taken together with the previous findings, our present data suggest that the blocking effects of diazepam on stress-induced activation of the mesoprefrontal DA neurons is likely related to specific anxiolytic action of BZDs mediated by BZD receptors. This suggestion is supported by the concomitant results of plasma corticosterone. Moreover, recent studies have provided considerable findings that the mesoprefrontal DA neurons in rats is selectively activated mediated through BZD receptors by FG 7142

(6,12), one of inverse agonists of BZD receptors, which have been reported to have anxiogenic effects in animals (9) and humans (2).

The present findings strongly support the hypothesis that the mesoprefrontal DA neurons play an important role in the control of negative emotional states (anxiety and/or fear) and that the blocking effect of BZDs on the hyperactivity of mesoprefrontal DA neurons may be one neural mechanism of their anxiolytic action.

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