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Pentobarbital Attenuates Stress-Induced Increases in Noradrenaline Release in Specific Brain Regions of Rats

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IDA, Y., A. TSUDA, S. TSUJIMARU, M. SATOH AND M. TANAKA. *Pentobarbital attenuates stress-induced increases in noradrenaline release in specific brain regions of rats.* PHARMACOL BIOCHEM BEHAV 36(4) 953–956, 1990.—To examine whether anxiolytic action of drugs acting at the GABA/BZD-chloride channel complex may be related to the brain noradrenergic system, we investigated the effect of pentobarbital, a typical barbiturate which has potent GABA modulating properties, on increased NA release in nine brain regions of stressed rats. Pentobarbital (10 and 25 mg/kg) was injected IP 65 min before sacrifice (5 min before one-hour immobilization stress). Levels of 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4), the major metabolite of brain noradrenaline (NA), and of plasma corticosterone, were fluorometrically determined. Pentobarbital treatment by itself increased MHPG-SO_4 levels in the thalamus, locus coeruleus (LC) region, midbrain and basal ganglia of nonstressed rats. Stress produced increases in MHPG-SO_4 levels in all brain regions examined and elevation of plasma corticosterone levels. Pentobarbital attenuated, in a dose-dependent manner, stress-induced increases in MHPG-SO_4 levels in the hypothalamus, thalamus, anterior cerebral cortex, LC region and basal ganglia and also attenuated the stress-induced elevation of plasma corticosterone levels. These data suggest that pentobarbital can attenuate both stress-induced increases in NA release in specific brain regions as well as activation of the hypothalamo-pituitary-adrenocortical system. These attenuating effects may be related to the anxiolytic action of barbiturates.

Pentobarbital Stress Noradrenaline release MHPG-SO_4 Rat brain regions Plasma corticosterone

A variety of stressful stimuli have been known to increase noradrenaline (NA) release in many brain regions in rats (7, 11, 24, 28, 29). We have reported that drugs with anxiolytic properties such as diazepam (8), ethanol (23) and opioid agents (26,27) show inhibitory effects on stress-induced increases in NA release in selected brain regions of rats, which may be closely related the the relief of hyperemotional (anxiety) responses during stress-exposure. As a result of these studies, we have proposed a hypothesis that increased brain NA release (mainly in the hypothalamus and amygdala) may have an important role in the provocation of aversive emotional changes (anxiety, fear or panic) of stressed animals.

Barbiturates, as well as benzodiazepines (BZDs) and ethanol, are thought to exert several pharmacological actions, including anxiolytic effects, by enhancing GABAergic inhibition via the GABA/BZD receptor-chloride channel complex (5). This raises the possibility that barbiturates might attenuate increases in NA release in brain regions of stressed rats following the interaction with GABA neurons. It has been reported that phenobarbital at a high dose (90 mg/kg) can block stress-induced increases in NA turnover in whole brain (14) and in the hypothalamus (12) of rats. However, there are few reports which have examined many brain regions of animals, wherein the metabolite levels were measured to determine the extent of NA release.

In the present study, by measuring levels of the major metabolite of rat brain NA, 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4), we investigated the effect of pentobarbital on stress-induced increases in NA release in nine brain regions of rats. Pentobarbital, a typical barbiturate which is more potent than phenobarbital (16) and GABA (15) at modulating the GABA/BZD receptor-chloride channel complex, was used at the dose range employed in behavioral studies (1, 5, 17, 18). Additionally, the drug effects on plasma corticosterone levels (a hormonal index of stress responses) were also examined.

METHOD

Animals

Male Wistar rats, weighing 170–190 g, were housed 4 per cage containing wood shavings at constant room temperature (24 ± 1°C) and humidity (50 ± 10%) and were allowed free access to food and water. The animal colony was maintained on a 12-hr alternating light-dark cycle with light on at 0700 hr. All experiments were

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carried out between 1000 hr and 1400 hr.

Drugs

Sodium pentobarbital (Sigma) was dissolved in saline and was injected IP in a fixed volume of 0.2 ml/100 g body weight.

Experimental Procedure

We used three separate rooms for injection, stress exposure, and decapitation. Forty-eight rats were randomly assigned to one of 6 groups of 8 rats each. On the experimental day, animals were injected IP with either pentobarbital at 10 or 25 mg/kg or saline 65 min before sacrifice, and those in three nonstress groups were returned to their home cages in the colony. Five min after injections, rats in the three stress groups were exposed to immobilization stress for one hour with a wire mesh (3×3 mm) by enclosing rats to fit to individual body size as reported previously (7,24).

Tissue Preparation and Biochemical Assay

Immediately after each experimental procedure, rats were sacrificed by decapitation. The brains were rapidly removed and were dissected on an ice-cooled plate according to the method of Gispen *et al.* (4) and frozen immediately on solid CO_2. Brain regions dissected were: the hypothalamus, amygdala, thalamus, hippocampus, cerebral cortex, pons, midbrain and basal ganglia. The locus coeruleus (LC) region was dissected from the pons by the method of Reis and Ross (22). The cerebral cortex was divided into anterior (mean weight 221 mg) and posterior (mean weight 310 mg) parts following transection of the vertical line through the optic chiasm and the anterior commissure, and these regions were examined individually. About 7 ml of blood from the cervical wound was collected into heparinized tubes (including 90 I.U. of heparin, each). Dissected brain tissues and separated plasma were stored at −80°C until assayed. Levels of MHPG-SO_4 were determined by our fluorometric method (13), which could detect 2 ng of MHPG-SO_4. Plasma corticosterone levels were determined fluorometrically by the method of van der Vies (30) with our slight modification, which could detect 80 ng of corticosterone. MHPG-SO_4 and corticosterone assays of 48 samples were performed at once, respectively.

Statistical Analysis

Statistical analyses for the data of each brain region and plasma corticosterone were made by a Stress (stressed and nonstressed) × Drug (pentobarbital 0, 10, and 25 mg/kg) factorial analysis of variance (ANOVA) and post hoc Newman-Keuls test for multiple comparisons, respectively. They were considered statistically significant when p value was equal or less than 0.05.

RESULTS

Figure 1 depicts the results of plasma corticosterone levels. ANOVA revealed a significant stress effect, $F(1,42)=74.5$, $p<0.01$, a significant drug effect, $F(2,42)=69.4$, $p<0.01$, and a significant interaction of stress × drug, $F(2,42)=49.1$, $p<0.01$. One-hour immobilization stress significantly increased plasma corticosterone levels. These increases were significantly attenuated by pretreatment with pentobarbital at 10 and 25 mg/kg.

Figure 2 shows the results of regional brain MHPG-SO_4 levels. ANOVA revealed a significant stress effect in the hypothalamus, $F(1,42)=69.4$, $p<0.01$, amygdala, $F(1,40)=41.3$, $p<0.01$, thalamus, $F(1,40)=5.4$, $p<0.05$, hippocampus, $F(1,41)=5.8$, $p<0.05$, anterior cerebral cortex, $F(1,39)=9.9$, $p<0.01$, poste-

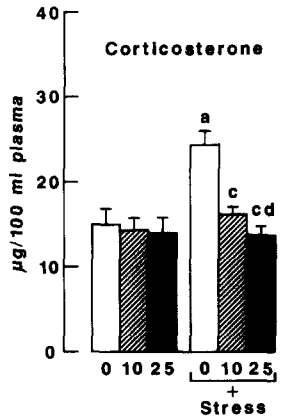

FIG. 1. Effect of pentobarbital on plasma corticosterone levels (μg/100 ml plasma) of stressed and nonstressed rats. Either pentobarbital at 10 mg/kg (hatched bars) or 25 mg/kg (solid bars) or saline (open bars) were treated IP 65 min before sacrifice (5 min before one-hour immobilization stress). Each value indicates the mean ± S.E.M. of 7–8 rats. The statistical significance (at least $p<0.05$) between two groups compared: a vs. saline control; c vs. stress + saline; d vs. stress + pentobarbital 10 mg/kg.

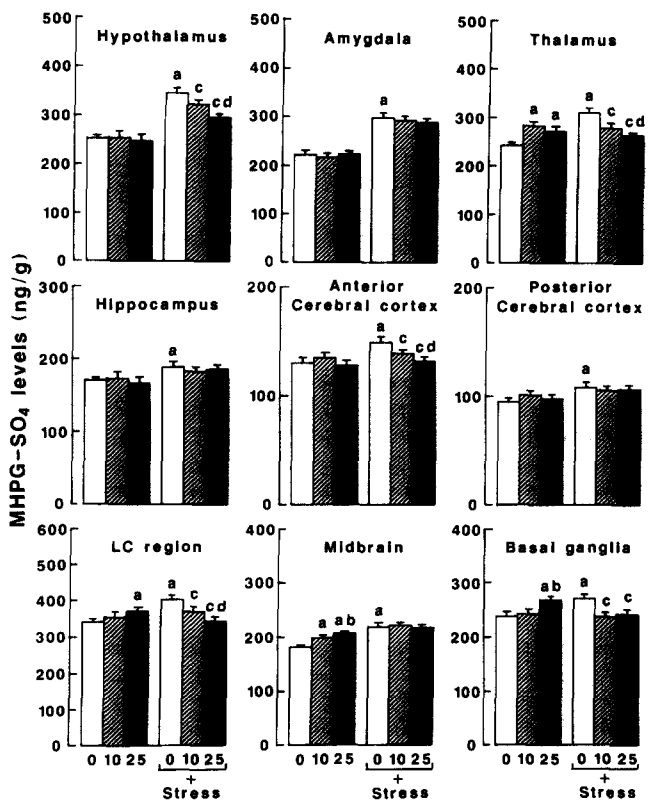

FIG. 2. Effect of pentobarbital on MHPG-SO_4 levels (ng/g) in nine brain regions of stressed and nonstressed rats. Either pentobarbital at 10 mg/kg (hatched bars) or 25 mg/kg (solid bars) or saline (open bars) were treated IP 65 min before sacrifice (5 min before one-hour immobilization stress). Each value indicates the mean ± S.E.M. of 7–8 rats. The statistical significance (at least $p<0.05$) between two groups compared: a vs. saline control; b vs. pentobarbital 10 mg/kg alone; c vs. stress + saline; d vs. stress + pentobarbital 10 mg/kg.

rior cerebral cortex, $F(1,40)=9.2$, $p<0.01$, LC region, $F(1,41)=4.3$, $p<0.05$, and midbrain, $F(1,41)=22.2$, $p<0.01$. There was a significant drug effect only in the basal ganglia, $F(2,41)=7.8$, $p<0.01$. ANOVA also showed a significant interaction of stress × drug in the thalamus, $F(2,40)=9.0$, $p<0.01$, and basal ganglia, $F(2,41)=15.1$, $p<0.01$. Pentobarbital at 10 and/or 25 mg/kg significantly increased MHPG-SO_4 levels in the thalamus, LC region, midbrain and basal ganglia. Immobilization stress for one-hour produced significant increases in MHPG-SO_4 levels in all brain regions examined. These increases in the metabolite levels were significantly attenuated by pretreatment with pentobarbital at 10 and 25 mg/kg in the hypothalamus, thalamus, anterior cerebral cortex, LC region and basal ganglia in a dose-dependent manner, but not in the amygdala, hippocampus, posterior cerebral cortex and midbrain.

DISCUSSION

The present study revealed that one-hour immobilization stress caused increases in MHPG-SO_4 levels in nine brain regions examined and plasma corticosterone levels of rats. These data are in agreement with previous reports (7,24), confirming that intense stress can elicit increases in NA release in extended brain regions and activate the hypothalamo-pituitary-adrenocortical system, a hormonal index of emotional (anxiety) responses.

Pentobarbital at 10 and 25 mg/kg attenuated stress-induced increases in NA release selectively in the hypothalamus, thalamus, anterior cerebral cortex, LC region and basal ganglia as well as activation of the hypothalamo-pituitary-adrenocortical system. These data are in agreement with previous findings indicating that phenobarbital prevents stress-induced acceleration of NA depletion by α-methyl-tyrosine, a tyrosine-hydroxylase inhibitor, in the neocortex including the hippocampus as well as in the rest of the brain (14), and that the same dose of phenobarbital reverses both depletion of NA levels in the hypothalamus as well as increases in plasma corticosterone levels caused by stress in rats (12). However, the present study is unique in that we now reveal the existence of regional differences in the attenuating effects of a barbiturate on stress-induced increases in brain NA release.

We have already reported that diazepam attenuates stress-induced increases in NA release in the hypothalamus, amygdala, hippocampus, cerebral cortex and LC region as well as distress-evoked hyperemotional responses such as defecation and vocalization via BZD receptors (8). Another series of our studies used opioid agents such as naloxone (25), morphine (26) and Met-enkephalin (27), and revealed that increases in NA release mediated by opioid receptors in the hypothalamus, amygdala and thalamus in rats may be related to the provocation of aversive hyperemotional responses observed during stress exposure. Furthermore, we have suggested that the selective attenuating effect of ethanol on stress-induced increases in NA release in the amygdala are closely related to the stress-relieving property of ethanol (23). Moreover, stressors without direct physical stimuli, such as conditioned fear (29) and a psychological stress model (11,29) can elicit increases in NA release selectively in the hypothalamus, amygdala and LC region. From these findings, we have proposed a hypothesis wherein stress-induced increases in regional NA release, especially in the hypothalamus and/or amygdala, may be closely related to the provocation of negative emotional responses elicited by stress, which may be biologically similar to anxiety, fear or panic reactions seen in humans. This hypothesis is supported by many studies in humans (2) and primates (21), which indicate that the brain noradrenergic system may play an important role in producing anxiety. When used at doses of less than 40 mg/kg, especially in the dose range from 5 to 15 mg/kg, pentobarbital has been reported to show anxiolytic properties with some sedation, in various behavioral studies (1, 5, 17, 18). Taken together with our previous studies, we suggest that the attenuating effect of pentobarbital on stress-induced increases in regional brain NA release, especially in the hypothalamus, may be related to the well-known anxiolytic action of barbiturates. The findings that pentobarbital could not attenuate stress-induced increases in NA release in the amygdala as well as the other three regions, might suggest that anxiolytic properties of barbiturates are not equivalent to those exerted by BZDs and ethanol.

Barbiturates are thought to exert anxiolytic action by enhancing GABAergic inhibition following acting at the chloride channel (barbiturate site) of the GABA/BZD receptor-chloride channel complex (6). We have already revealed that the BZD receptor-mediated attenuating effect of diazepam could be reversed by a GABA antagonist, picrotoxin, acting at the chloride channel site (9). Moreover, we have reported that FG 7142, an inverse agonist of BZD receptors which acts to reduce GABAergic inhibition, and which has anxiogenic effects in humans (3) and animals (19,20), can produce remarkable increases in brain NA release mediated through BZD receptors mainly in the hypothalamus and amygdala (10). Therefore, one neural mechanism of the anxiety- or stress-relieving properties of some drugs to modulate GABA transmission (including BZDs, ethanol and barbiturates) may be due to the prevention of increases in brain NA release, especially in the hypothalamus and/or amygdala as a consequence of their interaction with the GABA/BZD receptor-chloride channel complex.

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