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Effect of Diphenhydramine on Stress-Induced Changes in Brain Histidine Decarboxylase Activity, Histamine and Plasma Corticosterone Levels[1]

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MAZURKIEWICZ-KWILECKI, I. M. AND B. BIELKIEWICZ. *Effect of diphenhydramine on stress-induced changes in brain histidine decarboxylase activity, histamine and plasma corticosterone levels.* PHARMAC. BIOCHEM. BEHAV. **16**(4) 591–597, 1982.—Exposure of rats to platform stress induced a significant elevation in hypothalamic histamine levels. Air blast-stress resulted in a significant increase in hypothalamic histamine concentration and in histidine decarboxylase activity. No significant changes were noted either in the enzyme activity or in histamine levels in the midbrain or cortex of stressed rats. In the nonstressed rats, diphenhydramine (7.5 mg/kg intragastrically), a H_1-receptor antagonist, did not influence histidine decarboxylase activity or histamine concentration in any of the three brain regions investigated. However, diphenhydramine pretreatment prevented the increase in histidine decarboxylase activity induced by air blasts. In untreated rats, plasma corticosterone levels were significantly elevated following either platform stress (4.5-fold) or air blasts (7.8-fold). A significant increase was also noted in saline and diphenhydramine-treated animals following these stressors, however, the increase in saline or diphenhydramine treated rats following air blasts was significantly less than that seen in untreated stressed controls.

Histamine Histidine decarboxylase Brain Hypothalamus Stress Corticosterone Diphenhydramine Rats

DESPITE the rapidly accumulating evidence which suggests histamine as a central neurotransmitter or neuromodulator [2, 12, 16, 35–39, 43], the physiological role of this biogenic amine remains enigmatic. Both excitatory and inhibitory central effects were reported following microiontophoretic and microelectrophoretic histamine application [14, 15, 28, 32]. In line with these reports, central ascending histaminergic pathways have been implemented in the control of sleep and wakefulness [10,36] and circadian variations in hypothalamic histamine level have been demonstrated [26].

In view of these findings, the investigation of a possible involvement of brain histamine in response to stressful conditions was of interest. Only few and contradictory findings were reported following stress of restraint, cold exposure, or electric foot shock treatment applied to rats, mice or guinea pigs [3, 17, 21, 40, 46].

In our previous studies, a significant elevation in hypothalamic histamine levels in rats was observed following "platform stress" [24] or air blast exposure [25]. It was of interest to further investigate whether these alterations were related to concommitant changes in the histamine synthetizing enzyme, histidine decarboxylase. In addition, the suggested involvement of central H_1- and H_2-histaminergic receptors in ACTH regulation [34] and the recently demonstrated very potent antagonistic effects of diphenhydramine on central H_1-receptors [38] prompted us to explore the effects of this drug on stress-induced changes in hypothalamic HA concentration.

METHOD

Male Sprague-Dawley rats (200–220 g) were housed in plastic cages (4 rats per cage) in a temperature controlled room (22°C) with lights 7 a.m.–7 p.m. The animals were exposed to the "platform stress" in a procedure similar to that used in rapid eye movement (REM) sleep deprivation studies [4]. The rats were placed on a small circular platform (5 cm in diameter and 12 cm in height) centrally located in a round (50 cm in diameter) tub of water (24°C); the depth of water was 10 cm. The animals were exposed to this procedure for 15 minutes. Another group of nontreated-rats was subjected to 15 min of air blast stress [44]. The individual rats were placed

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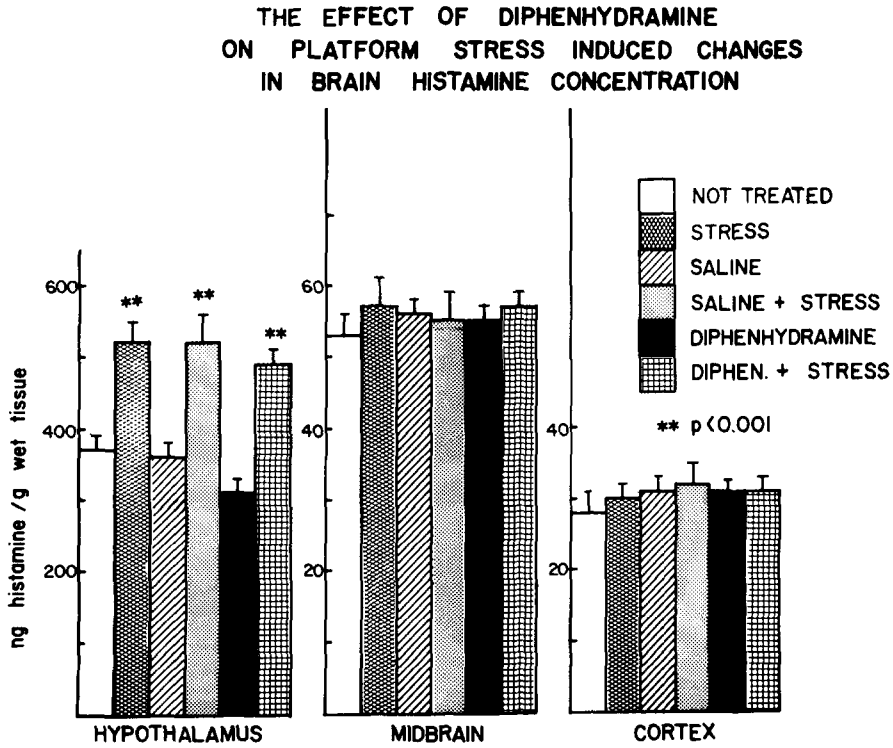

FIG. 1. Nontreated rats and diphenhydramine (7.5 mg/kg intragastrically, 1 hr before stress) or saline (0.9%) pretreated animals were exposed to 15 min of platform stress and sacrificed immediately after. The nonstressed, nontreated and diphenhydramine or saline-treated rats served as controls. The data represent the mean±S.E.M. of 12 experiments in each group of stressed and nonstressed rats. Statistical significance obtained by ANOVA and Duncan's test.

in a plastic cage (20.5×26.5×14.5 cm) and exposed to blasts of compressed air delivered from a nozzle placed 10 cm above the cage. The blasts were of 1 sec duration and were applied at different time intervals at a rate of 5/min. The last blast was always delivered at the end of the experimental period of time, i.e., at 15 min.

Another two groups of rats were administered diphenhydramine HCl, in a dose of 7.5 mg/kg intragastrically, 1 hour before the exposure to the platform stress or to the air blasts. Diphenhydramine treated rats subjected to the same handling but not exposed to stress served as controls. To account for the stress of drug administration, an additional third group of rats was administered 0.9% saline intragastrically and divided into nonstressed and stressed animals. All experiments, which included treated and nontreated rats, were carried out between 8:30 a.m. and 11:30 a.m. when plasma corticosterone is at a relatively low level [26].

The rats were sacrificed immediately after the stressful procedures, non stressed animals were decapitated at the same time. Following decapitation blood was collected from the severed neck blood vessels into heparin containing tubes for corticosterone determination. The brains were rapidly removed, washed with ice-cold saline, blotted and placed on glass plates kept on ice. Various brain regions were dissected according to a modified method of Glowinski and Iversen [11].

Histamine Determinations

The tissues were assayed for their histamine content according to a modification of the double isotope technique of Taylor and Snyder [41]. This procedure depends on the methylation of endogenous histamine in the tissues by added histamine N-methyltransferase, using S-adenosyl-L-methionine, methyl (^{14}C); (59 mCi/mmole, New England Nuclear) as the methyl donor. A tracer amount of 3-H-histamine (5–10 Ci/mmole, New England Nuclear) was added to correct for the varying degree of histamine methylation in different samples. Endogenous S-adenosylmethioninewas destroyed by boiling the tissue, a procedure which also served to precipitate protein.

The (^{14}C)-(^{3}H)-methylhistamine and (^{14}C)-methylhistamine were separated from (^{14}C)-S-adenosylmethionine and (^{3}H)-histamine be extracting into chloroform from a salt saturated sodium hydroxide solution. The chloroform was evaporated and ethanol and scintillation fluid (Econofluor) were added to the residue and counted in a Beckman LS 8100 liquid scintillation spectrometer.

Histidine Decarboxylase Assay

The histidine decarboxylase assay was determined by a modification of the method of Bielkiewicz [1,23]. The tissue samples were homogenized in 10 mM phosphate buffer (pH

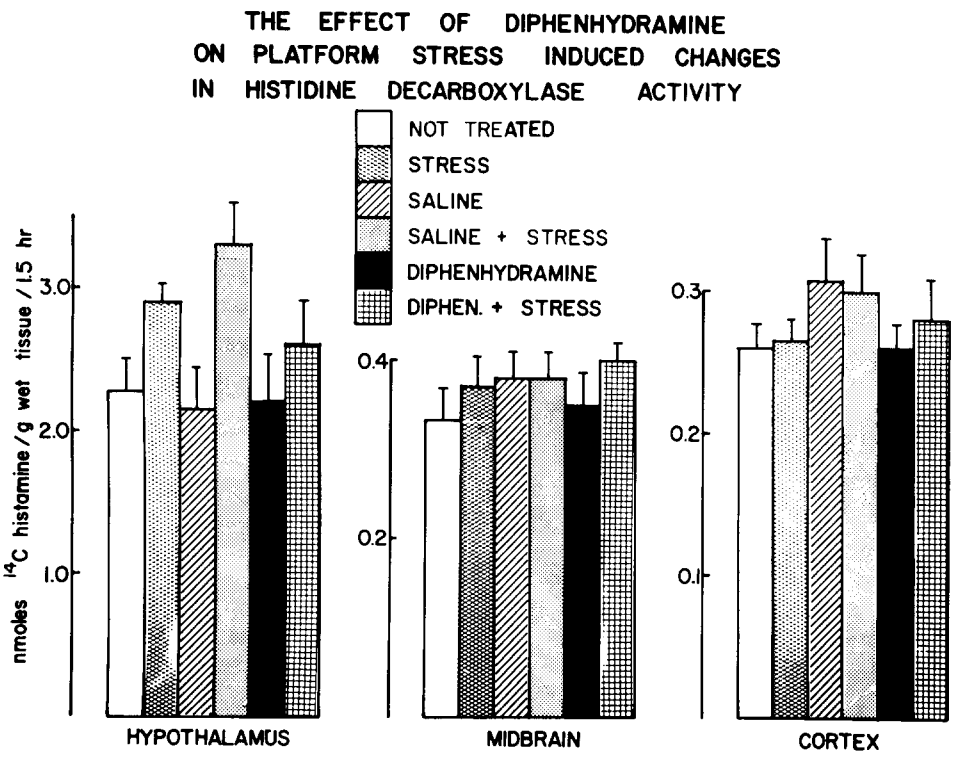

FIG. 2. Nontreated rats, and diphenhydramine (7.5 mg/kg intragastrically, 1 hr before stress) or saline (0.9%) pretreated animals were exposed to 15 min of platform stress and sacrificed immediately after. Nonstressed (nontreated and diphenhydramine or saline-treated) rats served as controls. The data represent the mean±S.E.M. of 6 experiments in each group of stressed and nonstressed rats.

7.0) and incubated for 1.5 hours at 37°C under nitrogen. The composition of the incubation mixture (0.4 ml) was similar to that reported previously [23] and consisted of 0.3 ml of homogenate, histamine dihydrochloride (10^{-4} M) in 10 mM phosphate buffer (pH 7.0), pyridoxal-5'-phosphate (10^{-6} M), aminoguanidine (10^{-6} M) and 0.4 μCi of ^{14}C-histidine (55 mCi/mmole, Amersham). Blanks contained the same composition except that homogenates were substituted by phosphate buffer (pH 7.0). At the end of the incubation period L-histidine (100 μg) was added as a carrier. The incubation was stopped after 1.5 hours by adding 50 μl of 1 N perchloric acid. The samples were then centrifuged for 10 min at 10,000 g. Part of the supernatant was counted for total radioactivity in a Beckman LS 8100 liquid scintillation spectrometer. Another part of the supernatant was subjected to high voltage electrophoresis (1500 V) in acetate formate buffer (pH 1.9) for 1.5 hours in order to separate ^{14}C-histamine from ^{14}C-histidine. Standard non-labelled histamine and L-histidine were used as markers and identified with ninhydrin. Electrophoretic strips of the corresponding labelled compounds were cut off, eluted with 1.5 ml of water, and after addition of 10 ml of Aquafluour (New England Nuclear) counted for the radioactivity. The data is reported in terms of nmol of ^{14}C-histamine per gram of wet tissue, formed within 1.5 hours of incubation period.

Plasma Corticosterone Assay

Plasma corticosterone concentration was determined by a modification of the method of Givner and Rochefort [10] which is based on the capability of corticosterone to fluoresce in sulfuric acid. The data is reported as μg per 100 ml of plasma (μg%).

The data was analyzed for statistical significance by two-way analysis of variance (ANOVA) and Duncan's Multiple Range test. The number of experiments is indicated in the legends to the figures.

RESULTS

In agreement with our previous report [24], platform stress (Fig. 1) induced again a significant (42%) elevation in hypothalamic histamine levels in nontreated rats. Saline or diphenhydramine-treated animals responded to stress in a similar manner; the increases were 45% and 58%, respectively, $F(1,2)=100.64$; $p<0.001$. Platform stress did not affect midbrain or cortical histamine levels of nontreated, saline-treated or diphenhydramine-administered rats.

There was no significant difference in hypothalamic histidine decarboxylase activity of nonstressed, saline or diphenhydramine-treated rats as demonstrated in Fig. 2. A two way ANOVA indicated that platform stress did not induce significant changes in hypothalamic histidine decarboxylase activity in any of the three groups tested, $F(1,2)=10.86$. Midbrain or cortical histidine decarboxylase activity was not significantly altered by platform stress in either nontreated, saline, or diphenhydramine-administered rats (Fig. 2).

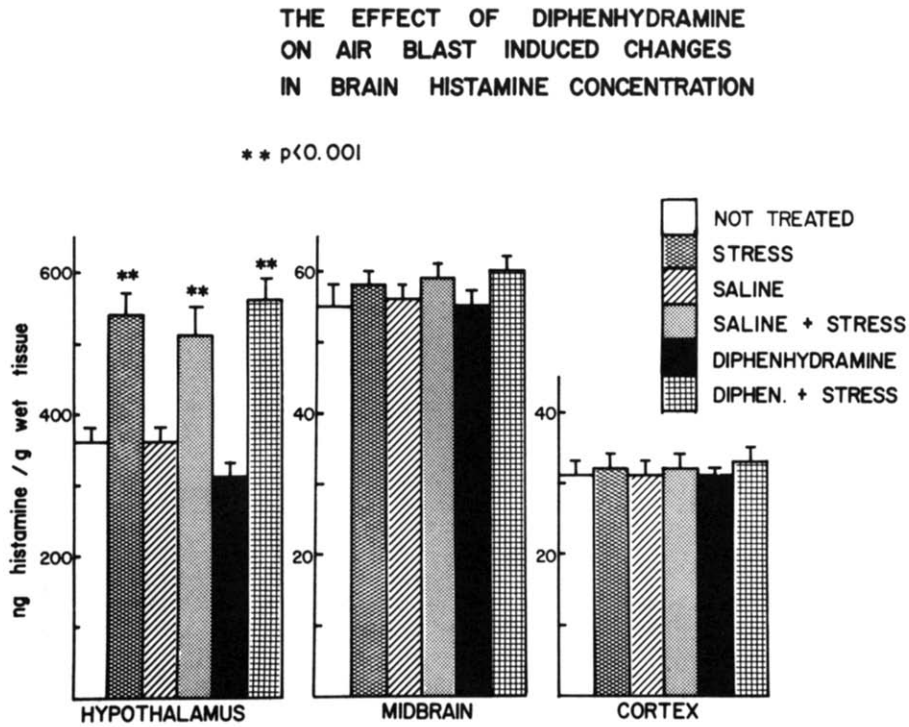

FIG. 3. Nontreated rats and diphenhydramine (7.5 mg/kg intragastrically, 1 hr before stress) or saline (0.9%) pretreated animals were exposed to 15 min of air blasts and sacrificed immediately after. Nonstressed (nontreated and diphenhydramine or saline-treated) rats served as controls. The data represent the mean±S.E.M. of 12 experiments in each group of stressed and nonstressed rats. Statistical significance obtained by ANOVA and Duncan's test.

Exposure to air blasts (Fig. 3) induced a significant elevation (50%) in hypothalamic histamine levels of nontreated rats but did not affect midbrain or cortical histamine concentration. This is in line with our previous report [25]. Saline or diphenhydramine-treated rats responded to air blasts also with a significant increase in their hypothalamic histamine levels, $F(1,2)=139.96$; $p<0.001$. The increase was not different from that seen in the nontreated rats (Fig. 3) Midbrain or cortical histamine concentration was not affected by air blast stress in any of the three groups investigated. Diphenhydramine treatment alone did not significantly alter hypothalamic, midbrain or cortical histmine levels.

Air blasts induced significant elevation in the hypothalamic histidine decarboxylase activity (Fig. 4) of nontreated (76%) and saline-treated (61%) rats, $F(2,42)=6.03$; $p<0.05$. However, no significant changes were noted in the diphenhydramine-pretreated rats. Air blasts did not alter histidine decarboxylase activity in the midbrain or cortex of nontreated, saline or diphenhydramine-administered rats. Diphenhydramine treatment alone was without any affect on hypothalamic, midbrain or cortical histidine decarboxylase activity (Fig. 4).

Plasma corticosterone levels were similar in all groups of nonstressed rats as indicated in Fig. 5A and B. Platform stress (Fig. 5A) induced a significant elevation in plasma corticosterone concentration in nontreated (4.9-fold) and in saline- (4.5-fold) or diphenhydramine- (4.3-fold) administered rats, $F(1,2)=683.41$; $p<0.001$.

Nontreated rats exposed to air blasts (Fig. 5) responded with a significantly greater increase in plasma corticosterone level (7.8-fold) than that noted in saline (4.3-fold) or diphenhydramine-administered stressed (4.2-fold) rats. The increases in plasma corticosterone in the latter two groups were significant and of similar magnitude, but significantly less than in untreated animals (Duncan's test). The values for stress were: $F(1,2)=1303.77$; $p<0.001$ and for drug treatment and stress interactions, $F(2,42)=25.10$; $p<0.001$.

DISCUSSION

Present data indicate that platform stress and air blast exposure induced significant elevation in hypothalamic histamine concentration. This increase was more evident following air blasts; the latter stress was also associated with higher plasma corticosterone levels. Also histidine decarboxylase activity was significantly increased following air blasts, while the incfease was less marked after platform stress. These results suggest that stress-induced increase in hypothalamic histamine levels may be due to an increased histamine synthesis. However, changes in the release and/or metabolism of histamine in stressed animals could have also contributed to the alterations in the hypothalamic histamine levels.

Other brain regions did not display stress-induced changes either in brain histamine levels, as we reported pre-

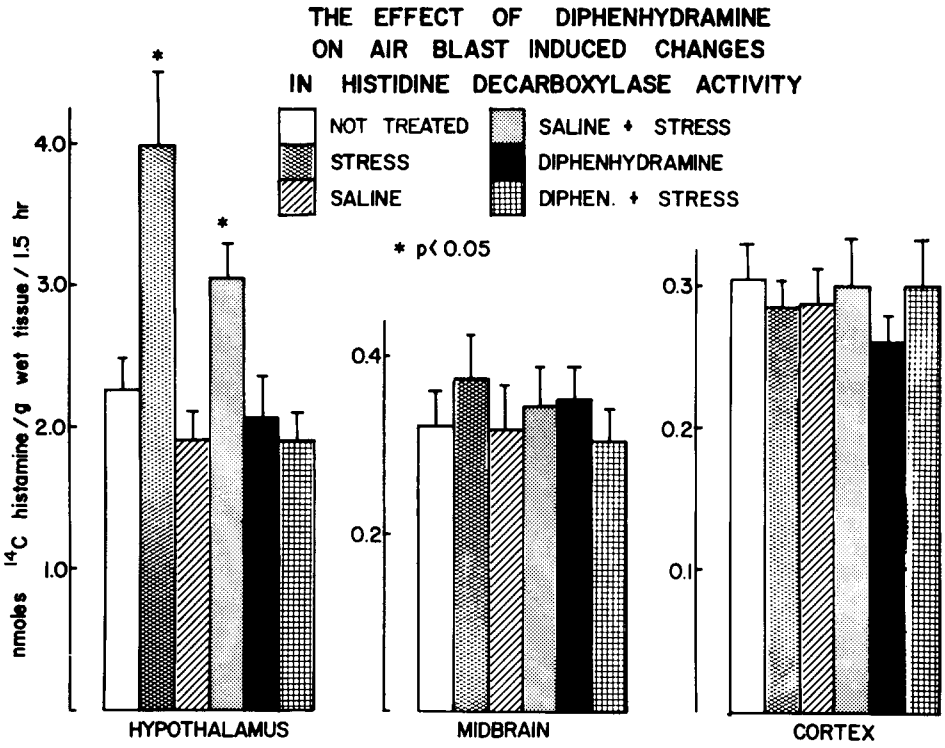

FIG. 4. Nontreated and diphenhydramine (7.5 mg/kg intragastrically, 1 hr before stress) or saline (0.9%) pretreated rats were exposed to 15 min of air blasts and sacrificed immediately after. Nonstressed (nontreated and diphenhydramine or saline-treated) rats served as controls. The data represent the mean±S.E.M. of 8 experiments in each group of stressed and nonstressed rats. Statistical significance obtained by ANOVA and Duncan's test.

viously [24,25], or in histidine decarboxylase activity which was determined in the present investigation.

It is of interest that stress-induced changes in histamine level and in histidine decarboxylase activity were noted only in the hypothalamus, a brain region known to play a significant role in the response to stress [7]. Also histamine turnover was indicated to be the most rapid in the hypothalamus. Many biochemical studies suggested a role of histamine in hypothalamic function [29, 31, 39, 45].

Due to the special type of stressors applied in the present investigation, our data is not readily comparable with other reports; stress-induced changes in brain histamine seem to depend on the type of stress applied and on the animal species. Electric foot shock increased histamine concentration in cerebral hemispheres and brain stem of rats [3] while an increased histidine decarboxylase activity was reported in guinea pig hypothalamus and cortex [21]. Also, an increased ^{3}H-histamine synthesis in the hypothalamus and in cortex was noted following cold exposure and restraint in rats although the histamine concentration in these brain regions was decreased [40]. The latter report could not be confirmed by others who did not find any changes following the same experimental conditions [17]. In immobilized mouse, brain ^{3}H-histamine turnover was reduced but histamine level remained unchanged [46]. Stress of hypoxia depressed hypothalamic histidine decarboxylase activity in male and female adult rats while no significant changes in histidine decarboxylase activity was noted in cortex, where histamine methyltransferase activity was increased [22].

In the present studies, acute diphenhydramine treatment alone did not significantly affect basal hypothalamic histamine levels, histidine decarboxylase activity or platform and air blast-induced elevation of hypothalamic histamine levels. However, diphenhydramine, which is a very potent central H_1-receptor antagonist [38], prevented air blast-induced increase in histidine decarboxylase activity.

This inhibition was not immediately reflected in the endogenous histamine levels possibly because the latter are controlled also by the activity of histamine methyltransferase; this enzyme was demonstrated to be inhibited by antihistaminic drugs *in vitro* studies [42].

Presently noted inhibitory effects of diphenhydramine on stress-induced changes in histidine decarboxylase activity are of interest in view of the suggested involvement of histamine in ACTH regulation [27]; H_1-receptor stimulation increased ACTH release and this effect was inhibited by H_1-receptor antagonist. H_2-receptor activation had inhibitory effects on ACTH release [34]. Our data may suggest that H_1-receptor activation could also be involved in stress-induced alterations in hypothalamic histamine regulation.

Plasma corticosterone elevation in response to stress was not affected by diphenhydramine. Others could not find any correlation between plasma corticosterone and brain histamine levels [46]. Our studies could not demonstrate a consis-

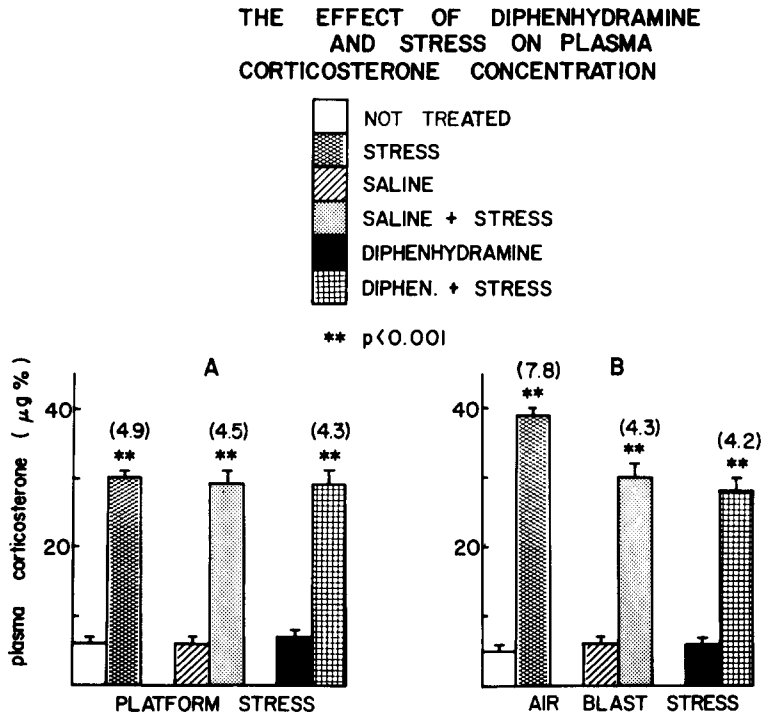

FIG. 5. Plasma corticosterone concentration of nontreated and diphenhydramine or saline-treated rats exposed to platform stress (A) or to air blasts (B). The respective nonstressed rats served as controls in each group. The data represent mean±S.E.M. of 10 experiments in each group of stressed or non stressed rats in (A) and 8 experiments in each group in (B). The numbers in brackets represent the ratio of stressed to nonstressed values. Statistical significance obtained by ANOVA and Duncan's test.

tent relationship between circadian variations in hypothalamic histamine levels and plasma corticosterone concentration [26]. We also indicated that in stress of longer duration (60 min) hypothalamic histamine concentration returned to control levels when plasma corticosterone levels were still elevated [24].

Centrally applied histamine affects prolactin release [6, 19, 30, 33]. It is possible that presently noted changes in hypothalamic histamine regulation following stress exposure could be linked also with stress-induced prolactin release which is also inhibited by H_1-receptor blockade by diphenhydramine [33].

Brain histamine was reported to play a role in physiological [12, 13, 18, 20] and behavioural processes [5,9]. Our data demonstrates that hypothalamic histamine regulation is also altered following exposure to different types of stressors. Air blast-induced increase in histidine decarboxylase activity is affected by H_1-receptor blockade.

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