Pharmacology Biochemistry & Behavior, Vol. 14, pp. 333–338. Printed in the U.S.A.

Effect of Diazepam on Stress Induced Changes in Brain Histamine[1]

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Received 5 August 1980

MAZURKIEWICZ-KWILECKI, I. M. *Effect of diazepam on stress induced changes in brain histamine.* PHARMAC. BIOCHEM. BEHAV. **14**(3) 333–338, 1981.—Acute treatment with diazepam (2.5, 5 and 10 mg/kg) did not affect the basal histamine concentration in the hypothalamus, midbrain or in the cortex of the rat. The increase in the hypothalamic histamine level caused by 15 min of "platform stress" was significantly attenuated by diazepam (5 or 10 mg/kg) pretreatment, but the elevation induced by 15 min of air blasts remained unchanged. Diazepam significantly reduced the rise in plasma corticosterone concentration in response to air blast stress but did not affect the increase caused by "platform stress." Thus, in addition to the already known effects of diazepam on stress induced changes in other central neurotransmitters or neuromodulators, diazepam may also affect the hypothalamic histamine elevation induced by certain type of stress.

Diazepam Stress Brain histamine

THE existence of central ascending histamine synthetizing neurones [1, 2, 6] and the effects of histamine on single neurones observed in microelectrophoretic and microelectrophysiological studies [12, 29, 54, 58] has added additional support to the suggested role of histamine as a possible central neurotransmitter or neuromodulator [8, 10, 28, 52, 59, 60, 61, 63]. Histamine sensitive adenylate cyclase has been found in both brain homogenates [30] and brain slices [5] and several studies have revealed the presence of cerebral H_1- and H_2-receptors [9, 14, 51]. H_1-receptor blocking agents have been known to induce sedative effects [22] while more recent investigations indicated the involvement of H_2-receptors in the mechanism of action of tricyclic antidepressants [34,55]. Brain histamine was found to influence thermoregulation [27,44], emesis [7], water intake [42], self-stimulation [16] and avoidance behavior [24]. In addition, histamine induced release of vasopressin [19], prolactin [20, 43, 53, 56] and ACTH has been demonstrated [57]. Despite these numerous reports the functional role of brain histamine is not yet clearly understood.

We reported previously [48,49] that different types of short lasting stress induced significant alterations in brain histamine concentration of several brain regions of the rat. The present investigation was undertaken in order to further explore the mechanism of these changes. Because anxiety is usually associated with unpleasant stressful conditions it was of interest to investigate whether the widely used anxiolytic drug, diazepam, could affect stress induced alterations in brain histamine. Diazepam has been reported to modify stress-related changes in the level of several central neurotransmitters and neuromodulators [23,64], however, the exact mechanisms by which it produces its effects is still unclear; moreover, diazepam-histamine interactions were little explored.

METHOD

Male Sprague-Dawley rats (200–220 g) were housed in plastic cages (3 rats per cage) in a temperature controlled room (22°C) with lights 7 a.m.–7 p.m. One group of animals was exposed to the "platform stress" in a procedure similar to that used in rapid eye movement (R.E.M.) sleep deprivation studies [15]. The rats were placed on a small circular platform (5 cm in diameter and 12 cm in height) centrally located in a 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 experimental rats was subjected to 15 min of air-blast stress. The individual rats were placed in a plastic cage (20.5×26.5×14.5 cm) and exposed to blasts of compressed air [69] delivered from a nozzle placed 10 cm above the cage. The blasts were of 1 second 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 minutes. Diazepam was administered in doses of 2.5, 5 and 10 mg/kg orally 1 hour before the exposure to the platform stress or to the air blasts. Diazepam treated rats subjected to the same handling but not exposed to stress served as controls. All experiments were carried out between 8:30 a.m. to 11:30 p.m. when plasma corticosterone is at a relatively low level [50]. The absence of any effects of the vehicle on the basal brain histamine levels and on plasma corticosterone concentration was established in

[1]This research was supported by Ontario Mental Health Foundation grant no. 789;80-82

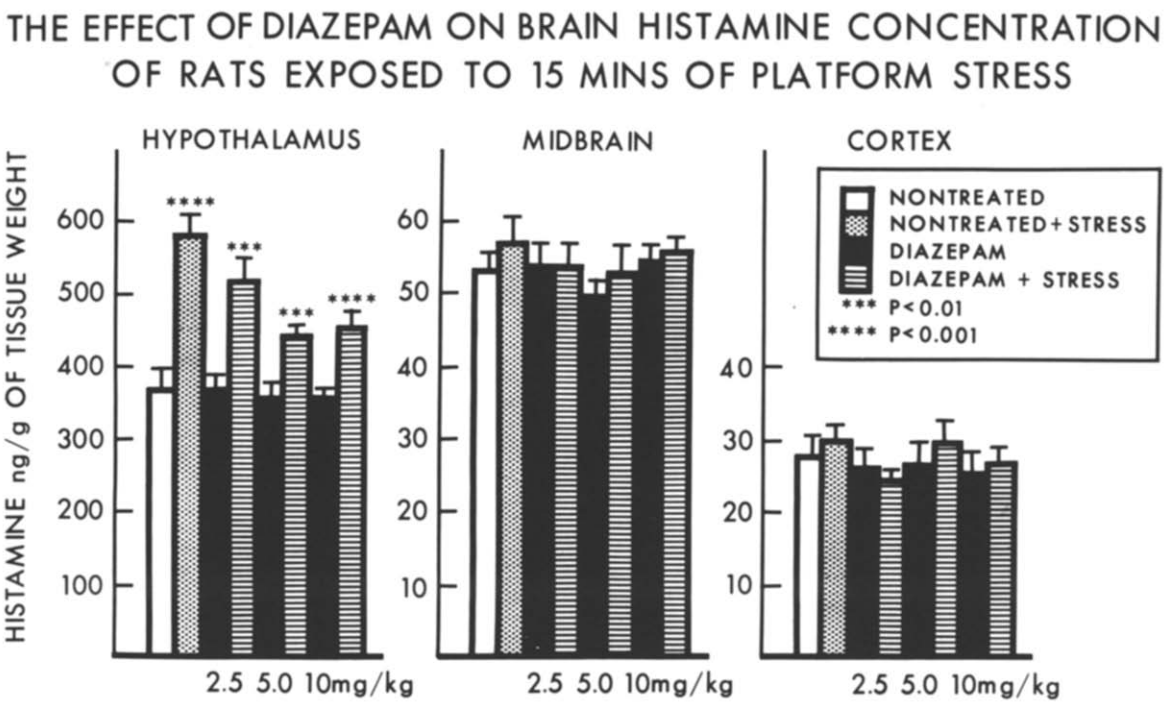

FIG. 1. Diazepam pretreated rats (2.5, 5 or 10 mg/kg orally, 1 hour before stress) were exposed to platform stress (see Method) for 15 min and sacrificed immediately after. Diazepam treated nonstressed rats served as controls. The data represent the mean ± S.E.M. of 8–12 experiments. Stress induced changes in brain histamine levels of nontreated rats are enclosed for the sake of comparison.

preliminary experiments. The vehicle consisted of lactose, corn starch and magnesium stearate and was supplied by Hoffman-La Roche, Ltd.

Experimental rats were sacrificed immediately after the stressful procedures. Controls, nonstressed 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. Different brain regions were dissected according to modified method of Glowinski and Iversen [26].

Histamine Determinations

The tissues were assayed for their histamine content according to a modification of the double isotope technique of Taylor and Snyder [66]. This procedure depended on the methylation of endogenous histamine in the tissues by added histamine methyltransferase, using S-adenosyl-L-methionine methyl (^{14}C); (56 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-adenosylmethionine was 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 by extracting into chloroform from a salt saturated alkaline solution. The chloroform was evaporated and the residue was taken up into ethanol and scintillation fluid (Econofluor) and counted in a Beckman LS 8100 liquid scintillation spectrometer.

Plasma Corticosterone

Plasma corticosterone was determined by a modification of the method of Givner and Rochefort [25] which is based on the capability of corticosterone to fluorescence in sulfuric acid. The data is reported as μg per 100 ml of plasma (μg%).

RESULTS

Diazepam vehicle did not affect the endogenous histamine concentration in any of the three brain regions investigated when tested 1 hour following treatment. Also plasma corticosterone level of vehicle treated rats although increased (10.1±2.5 μg%) did not significantly differ from that of nontreated controls (6.2±0.9 μg%). As indicated in Figs. 1 and 3, diazepam in the dose range tested did not alter the endogenous histamine levels in the hypothalamus, midbrain and in the cortex of nonstressed rats, and the basal plasma corticosterone concentration remained unaffected. The increase in hypothalamic histamine induced by 15 min of platform stress was significantly attenuated by the middle and the highest doses of diazepam (Fig. 1). While in the nontreated rats the elevation of hypothalamic histamine was 57%; in diazepam pretreated animals it was 20 and 28% following 5 and 10 mg/kg respectively. No significant effects were noted in the midbrain or cortical histamine levels of diazepam pretreated stressed rats.

In accordance with our previous observations [49] 15 min of air blast stress also resulted in a significant increase (41%) in hypothalamic histamine level (Fig. 2). In contrast to

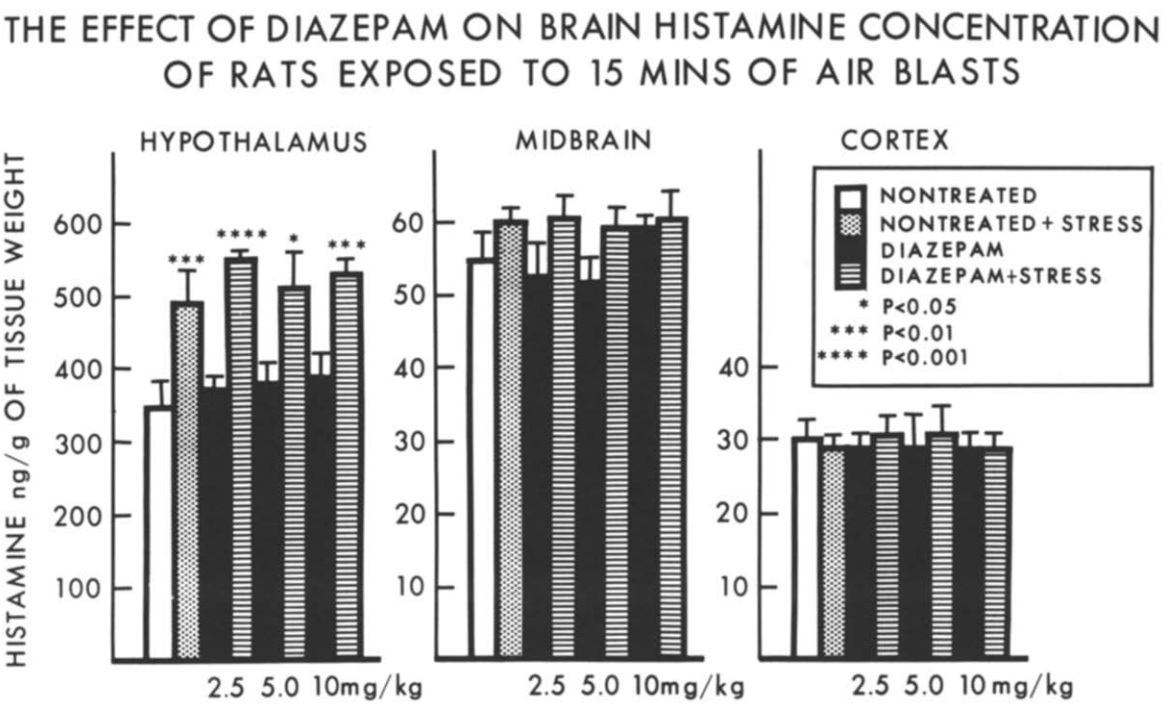

FIG. 2. Diazepam pretreated rats (2.5, 5 or 10 mg/kg orally, 1 hour before stress) were exposed to air blasts (see Method) for 15 min and sacrificed immediately after. Diazepam treated, nonstressed rats served as controls. The data represent the mean ± S.E.M. of 8–12 experiments. Stress induced changes in brain histamine levels of nontreated rats are enclosed for the sake of comparison.

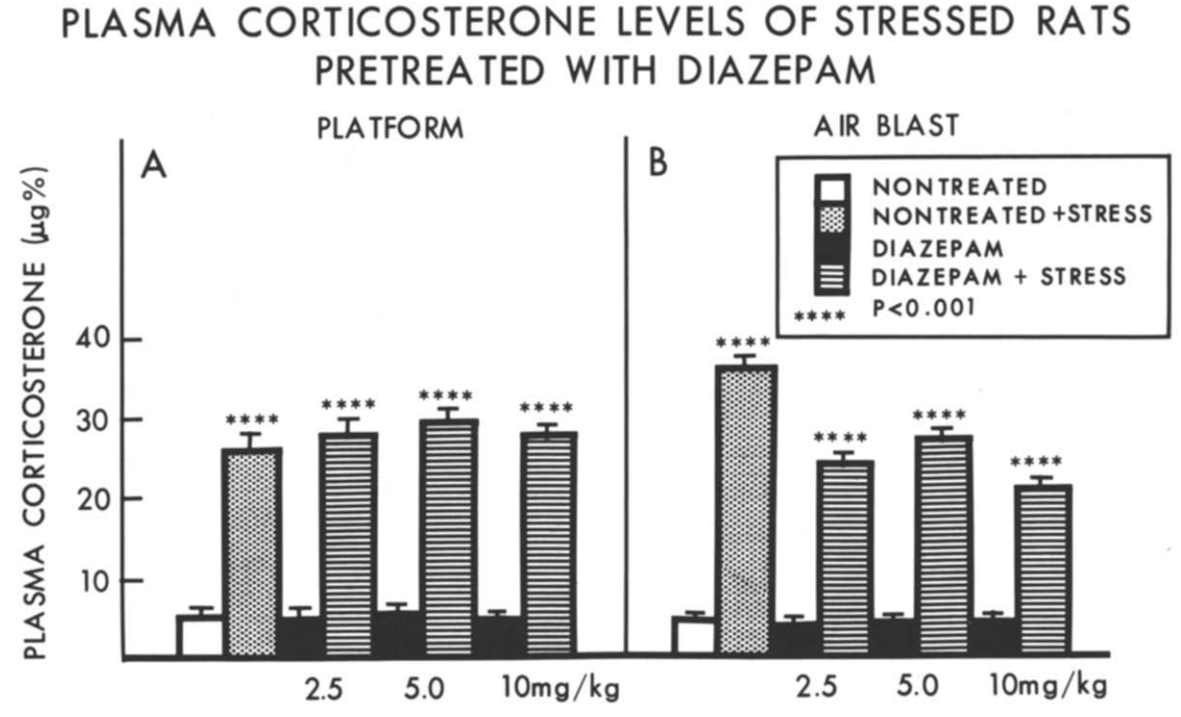

FIG. 3. Plasma corticosterone concentration in diazepam pretreated rats exposed to 15 min of platform stress (A) or to 15 min of air blasts (B). Diazepam treated, nonstressed rats served as controls. The data represent the mean ± S.E.M. of 8–12 experiments. Stress induced changes in plasma corticosterone levels of nontreated rats are enclosed for the sake of comparison.

the attenuating effects of diazepam on hypothalamic histamine elevation induced by platform exposure, the increase in histamine concentration induced by air blasts remained unaffected by pretreatment with 2.5, 5 and 10 mg/kg of diazepam (47, 32 and 35%, respectively). No significant alterations were noted in the midbrain and cortical histamine levels in diazepam-pretreated rats exposed to air blasts.

Platform stress (Fig. 3A) induced a significant elevation in plasma corticosterone concentration (26±2 μg%) of nontreated rats, as reported previously [48]. This rise was not affected by diazepam pretreatment.

In nonpretreated rats 15 min of air blast stress (Fig. 3B) induced a greater rise in plasma corticosterone (36.2±0.9 μg%) than that seen in rats exposed to 15 min of platform stress (26±2 μg%). Diazepam pretreatment significantly attenuated the plasma corticosterone elevation in response to air blasts. The plasma corticosterone levels were 23.6±2.8, 28.2±1.0 and 21.1±1.4 μg% following 2.5, 5 and 10 mg/kg of diazepam respectively. Although the largest dose of diazepam resulted in a greatest attenuation a strict dose-response relationship was not observed.

DISCUSSION

In confirmation with our previously reported observation [48,49], platform stress or air blast exposure induced a significant elevation in the endogenous histamine concentration in the hypothalamus, a brain region which plays an important role in the response to stress [21] and where histamine turnover is the most rapid [63]. The observed alteration in hypothalamic histamine level suggested an imbalance between the rate of synthesis and metabolism or disappearance of this biogenic amine.

Others found also increased histamine levels in the central hemispheres and in the brain stem of rats exposed to short lasting electric shock [11]. The reported decrease in hypothalamic histamine concentration induced by long periods (1–2 hours) of restraint or cold exposure [63] could not be confirmed [37]. Whole brain histamine turnover was decreased and brain histamine concentration remained unaltered in the restrained mice [70] while in guinea pigs electric shock treatment decreased whole brain histamine level [47]. However, the latter reports are not readily comparable with ours because of different type of stress applied, different time of sampling and different species used.

It should be noted that brain norepinephrine levels are also variably affected depending on the type of stressors used; thus grid shock raised hypothalamic norepinephrine concentration, whereas restraint reduced it significantly and acute exposure to new environment had minimal diminishing effects [35].

It is of interest that histamine synthetizing neurones in the middle forebrain bundle which spread widely in the whole ipsilateral telencephalon [61,62] were suggested to possibly influence arousal mechanism and to be affected during stressful situations [70]. Our own studies and that of others indicated circadian variations in hypothalamic histamine level which were inversely related to spontaneous locomotor activity [50,60] and temperature [50] suggesting a possible involvement of brain histamine in the states of activity and sleep. However, the physiological significance of altered brain histamine levels is not yet known. The increase in brain histamine concentration in mice, achieved by L-histidine loading, was associated with a significant decrease in motor activity, and depression of the fighting behaviour induced by electric footshock [18]. Intraventricularly administered histamine was found to reduce locomotor activity and in high doses to depress exploratory activity in rats [33]. Thus elevated levels of brain histamine seem to be associated with CNS depression. The behavioural effects of decreased brain histamine levels induced by α-hydrazino histidine administration in mice or rats were not investigated [65,70].

The increase in hypothalamic histamine concentration induced by platform stress was significantly attenuated by diazepam. Interestingly enough, acute treatment with diazepam (10 mg/kg, 3 hours before death) was reported to prevent the reduction in hypothalamic histamine level induced by restraint or cold exposure for 2 hours [67]. It may be noted that diazepam also counteracted changes in norepinephrine and dopamine level or turnover in the rat induced by electroshock treatment or immobilization stress [17, 36, 41].

In contrast to the significant attenuating effects of diazepam on hypothalamic histamine elevation induced by platform stress, the increase caused by air blast remained unchanged. On the basis of the elevation in plasma corticosterone levels, the latter procedure seemed to be more stressful to the rats and, as noted previously, changes in brain histamine level occurred more rapidly [49]. It is possible that higher doses of diazepam than the ones presently administered could have elicited an attenuating effect, however, large doses were reported to induce ataxic effects and to increase plasma corticosterone levels [40] and were therefore not investigated.

Diazepam in the range of doses tested presently did not affect the basal plasma corticosterone levels of nonstressed rats. Also, others [36] did not observe any significant alterations following somewhat smaller oral doses of diazepam. The reported increase in plasma corticosterone levels of rats following diazepam treatment could have been due to the parenteral route of administration [36,45], different time intervals before sampling [45], the use of anaesthesia [3,40] or due to larger doses applied [40].

Present studies indicated that the increase in plasma corticosterone induced by platform stress was not affected by diazepam but the elevation caused by air blasts was significantly reduced. In agreement with our latter observations, diazepam, in similar doses to the one presently used, attenuated the increase in plasma corticosterone induced by exposure to white noise [3], 30 min restraint [36], application of electric current [4,38] or exposure to novel environment with noise and sham IP injections [39]. The attenuating effects were suggested to be centrally mediated [36,39]. However, the rise in plasma corticosterone concentration caused by 30 min of footshock or 60 min of immobilization stress was not affected by diazepam pretreatment [3].

Despite the reported histamine-corticosterone interactions in the peripheral tissues [13, 31, 32, 46, 68] and histamine induced increase in ACTH secretion which was abolished by H_1-receptor blockade [57], this investigation could not establish a correlation between the increased plasma corticosterone level and hypothalamic histamine alterations. While diazepam attenuated platform stress induced elevation in hypothalamic histamine, the plasma corticosterone response remained unaffected. On the other hand diazepam did not influence the increase in hypothalamic histamine caused by air blast exposure but significantly reduced the rise in plasma corticosterone induced by this type of stress. Also, in our previous studies, despite the maintenance of significant elevation in plasma corticosterone levels following prolonged platform stress (60 min) or

air blasts (30 min) exposure, the hypothalamic histamine concentration returned to the basal values [48,49]. In line with our present observations, a significant elevation in plasma corticosterone levels induced by restraint in mice, was associated with unchanged brain histamine concentration [70]. Furthermore a decreased brain histamine level (induced by α-hydrazino-histidine administration) in mice resulted in an elevation of plasma corticosterone concentration, but an increase in whole brain histamine (following treatment with L-histidine) did not affect plasma corticosterone level [70].

Despite intensive investigation the exact mechanism of the CNS effects of diazepam is still far from being fully understood; 5-hydroxytryptamine, acetylcholine, glycine and γ-aminobutyric acid may all be involved [23,64]. Present studies indicated the attenuating effects of diazepam on platform stress-induced elevation in hypothalamic histamine level.

ACKNOWLEDGEMENTS

The technical assistance of Mr. Philander Baddoo is greatly appreciated. I also wish to thank Dr. M. Saucier and Dr. R. Phillips, Hoffman-La Roche Ltd. for the generous supply of diazepam.

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