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Single and Repeated Air Blast Stress and Brain Histamine[1]

I.M. MAZURKIEWICZ-KWILECKI

Department of Pharmacology, Faculty of Health Sciences, School of Medicine, University of Ottawa, Ottawa, Ontario, Canada K1N 9A9

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MAZURKIEWICZ-KWILECKI, I. M. *Single and repeated air blast stress and brain histamine*. PHARMAC. BIOCHEM. BEHAV. 12(1) 35–39, 1980.—Exposure of rats to air blasts for 1, 5 and 15 min resulted in a significant increase in plasma corticosterone level and in the hypothalamic histamine concentration. Midbrain histamine content was increased after 1 and 5 min of exposure but cortical histamine increased following 1 min of exposure only. Stress of longer duration (30 mins did not significantly affect histamine concentration in any of the three brain regions investigated, although plasma corticosterone level remained very significantly (14.5-fold) elevated. Repeated exposure of rats to air blasts of 15 min duration resulted in a significant elevation of hypothalamic histamine concentration while midbrain and cortical histamine was not significantly altered. Plasma corticosterone level was again very significantly (10-fold) increased. Present results suggest the involvement of brain histamine in the response to stress.

Histamine Brain Stress Rat Corticosterone

STRESS has been used as a valuable tool to investigate the physiological role of central neurotransmitters. This subject was extensively reviewed [1, 17, 18, 36]. In recent years histamine has gained recognition as a putative central neurotransmitter [30–33, 37]. The existence of a central ascending histaminergic pathway which could be involved in control of sleep and wakefulness has been suggested [10,33] and histamine afferents to the hippocampal region were demonstrated [2]. In contrast to the numerous studies on the role of other biogenic amines in stressful conditions, the involvement of brain histamine has been relatively little explored. Conflicting results were reported when rats, mice or guinea pigs were restrained, exposed to cold or subjected to shock treatment [3, 21, 37, 41]. Our previous studies [22] have revealed a significant elevation in hypothalamic histamine concentration following stressful conditions with an emotional rather than physical component. Some adaptation to this response occurred following repeated exposure. The present investigation was undertaken in order to explore whether the previously observed alterations in brain histamine were specific to only one type of stress or could also occur in response to other stressful stimuli.

METHOD

Male Sprague-Dawley rats (200–220 g) were housed in metal cages (3 rats per cage) in a temperature controlled room (24°C) with lights 7 a.m.–7 p.m. In "acute" stress studies 4 groups of rats were used. Each group was exposed to either 1, 5, 15 or 30 min of stress. The stress was applied to individual rats placed in a plastic cage (20.5×26.5×14.5 cm) and consisted of exposure to blasts of compressed air [40] delivered from a nozzle placed 10 cm above the cage. The blasts were of 1 second duration and were delivered at different time intervals at a rate of 5/minute. The last blast was always applied at the end of the experimental period of time, i.e. at 1, 5, 15 or 30 min. The animals were sacrificed by decapitation immediately after the stressing procedure. Control rats were placed in individual cages of the same size and kept under identical experimental conditions but they were not subjected to stress.

In another set of experiments rats were exposed to air blast stress for 15 min twice for 4 days and sacrificed immediately after the last (eighth) 15 min exposure. Rats subjected to the same handling but not to stress served as controls. 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 a modified method of Glowinski and Iversen [13].

Histamine Determination

The tissues were assayed for their histamine content according to a modification of the double isotope technique of Taylor and Snyder [38]. This procedure depended on the methylation of endogenous histamine in the tissues by added histamine methyltransferase, using S-adenosyl-L-methio-

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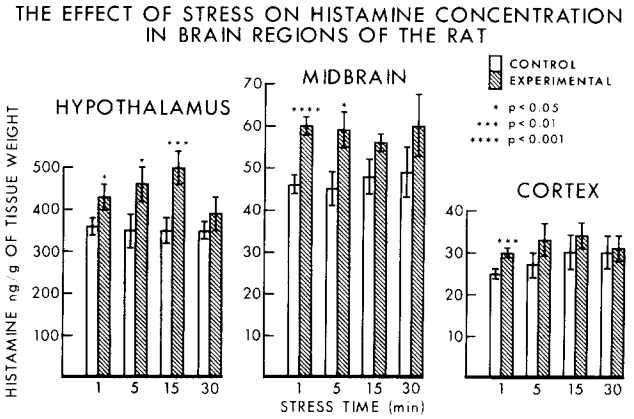

FIG. 1. Time course of stress induced changes in histamine concentration in different brain regions of the rat. The animals were exposed to air blasts for 1, 5, 15 and 30 mins and sacrificed immediately after. Non-stressed rats served as controls. The data represent the mean ± SEM of at least 8 animals in each group. *$p<0.05$; ***$p<0.01$; ****$p<0.001$.

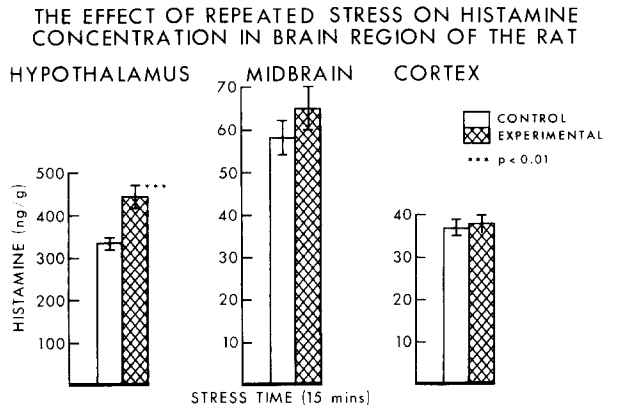

FIG. 3. The effect of repeated stress (15 min twice daily tor 4 days) on histamine concentration in brain regions of the rat. The data represent the mean ± SEM of 8 experiments, ***$p<0.01$ compared to control values.

nine 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 [12] which is based

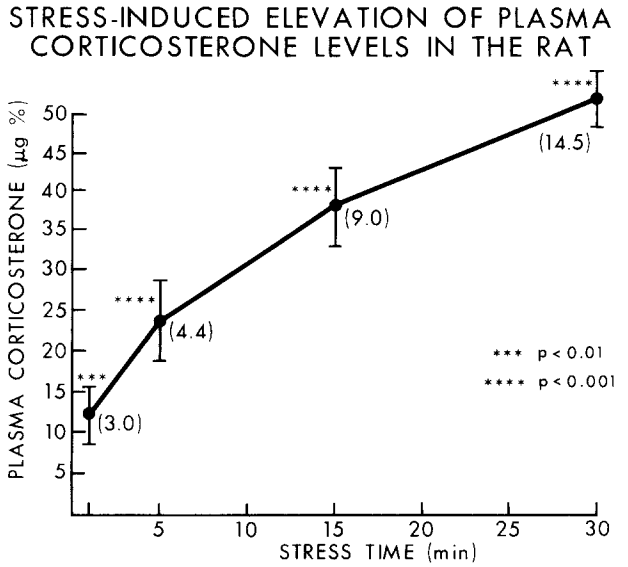

FIG. 2. Time course of stress induced elevation of plasma corticosterone concentration in the rat: μg% refers to μg100 ml of plasma. Poststress to prestress ratios are indicated in the brackets. The data represent mean ± SEM of 8 animals in each group. ***$p<0.01$; ****$p<0.001$.

on the capability of corticosterone to fluoresce in sulfuric acid. The data is reported as μg per 100 ml of plasma (μg%).

RESULTS

Exposure of rats to air blast for 1, 5 and 15 min resulted in a significant increase (19, 31 and 46% respectively) in the hypothalamic histamine concentration (Fig. 1). No significant change was noted following 30 mins of exposure.

Midbrain histamine concentration was significantly increased following 1 min (30%) and 5 minutes (31%) exposure to stress. Longer exposures resulted in an increase in the midbrain histamine level which did not reach the level of statistical significance.

A significant increase (20%) was also seen in cortical histamine level following 1 min exposure while longer periods of stressful stimulation did not significantly alter histamine level in this brain region.

The time course of stress induced elevation in plasma corticosterone level is indicated in Fig. 2. A significant (3-fold) elevation was noted following 1 min exposure to air blasts. Progressively higher values were seen following 5, 15 and 30 mins of stress. The values for poststress to prestress ratios were 3.0, 4.4, 9.0 and 14.5 for 1, 5, 15 and 30 min of exposure, respectively.

Repeated exposure of rats to the same type of stress (15 min of air blasts twice daily for 4 days) resulted in a significant (34%) elevation of hypothalamic histamine concentration, while no significant alterations occurred in the midbrain and cortical histamine level (Fig. 3). Plasma corticosterone level (Fig. 4) was again significantly (10-fold) elevated similarly to the increase (9-fold) noted in acute studies following a single exposure to 15 min of air blasting.

DISCUSSION

In this investigation stress induced by the exposure of rats

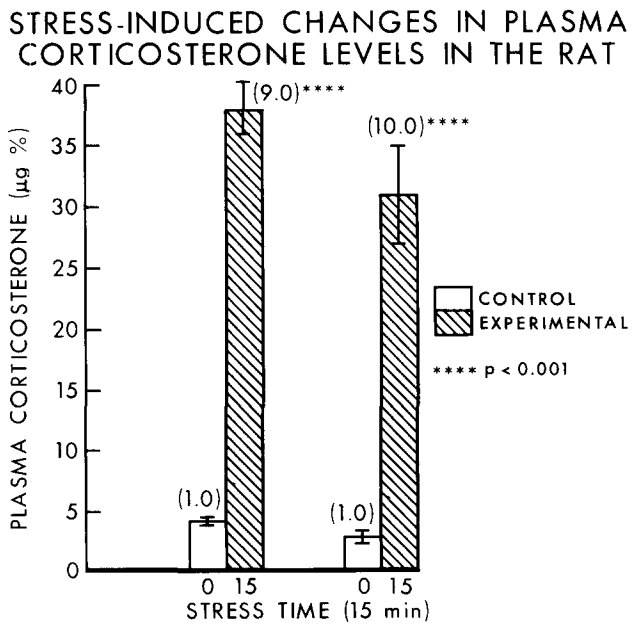

FIG. 4. Plasma corticosterone elevation after single exposure (15 min) to air blasts (A) and (B) following repeated exposure (15 min twice daily for 4 days); µg% refers to µg/100 ml of plasma. Post-stress to prestress ratios are indicated in brackets. The data represents the mean ± SEM of 8 experiments in each case. ****$p<0.001$ compared to control values.

to air blasts for different periods of time was associated with a significant increase in plasma corticosterone level which was tripled after one minute of exposure and kept rapidly rising with longer periods of stressful stimulation. On the basis of plasma corticosterone level this procedure seemed to be more stressful than the one applied in our previous studies [22]. In the latter investigation the rats were exposed to platform stress according to the method used in rapid eye movement (REM) deprivation studies [4] which consisted of placing the animal on a small platform (2 cm in diameter) located 2 cm above water (25°C), 10 cm deep.

In line with our previous observations [22] stressful stimulation which was applied in these studies was associated with a significant increase in hypothalamic histamine concentration; however, this effect was noted after one minute of exposure while in "platform stress" studies 15 min elapsed before such a change was observed. Midbrain histamine level was also significantly elevated following 1 or 5 min exposure to air blasts and cortical histamine concentration was increased following 1 min of exposure to stress. In contrast following "platform stress" a significant decrease in midbrain histamine level was noted following 5 mins of exposure and cortical histamine concentration was not significantly altered [22].

It is of interest that the most significant increases were observed again in the hypothalamus, a brain region whose role in response to stress was indicated [8], and where the highest concentration of histamine were found, and the most rapid histamine turnover has been reported [37]. This increase can be attributed to the stressful situation because normal values were found in control rats kept under identical experimental conditions but not subjected to stress.

The presently noted increase in the hypothalamic histamine level indicates an imbalance between histamine synthesis and metabolism and may possibly suggest a decreased rate of histamine disappearance or increased rate of synthesis.

Unfortunately, histamine turnover could not have been investigated under our experimental condition as even a single injection of histamine precursor, histidine has been reported to represent a considerable stress reflected in a significant elevation of plasma corticosterone level [41].

Our data is in agreement with the reported increase in histamine concentration in the central hemispheres and in the brain stem of rats following short lasting electric shock [3]. Present results indicate that stress induced changes in brain histamine occur rather rapidly and may not be detectable when the time of sampling is delayed. This is in line with the very rapid turnover of brain histamine in the non-mast cell pool [6,32].

In contrast to our observations a decreased hypothalamic histamine level was reported in rats following restraint or cold exposure for 1–2 hours [37], although the latter results were not confirmed by others using the same strain of rats and identical experimental conditions [16]. It is possible that hypothalamic histamine synthesis is increased initially but during longer periods of stress the release or utilization exceeds the synthesis and thus the endogenous levels fall. Long periods of exposure, the strong physical component in immobilization stress and metabolic factors involved in cold exposure, could account for the discrepancies between the results obtained by others and our data.

A decrease in brain histamine (determined by bioassay) was found in guinea pig following electric shock treatment [21] while in restrained mice brain histamine concentration remained unchanged but histamine turnover was decreased [41]. However, whole brain histamine levels were determined in the latter studies and possible individual opposite changes in different brain regions could have been left undiscovered.

In view of the different types of stress applied and various times of sampling, the reported data is not readily comparable with ours. Species differences may also exist.

Similar discrepancies occur in the literature on the effects of various forms of stress on catecholamine levels. Thus rats subjected to acute immobilization stress, cold or formalin injections showed decreased norepinephrine and/or epinephrine concentrations in specific hypothalamic nuclei [17–19] or brain stem areas [28]. In addition, footshock, treadmill, swim or restraint also resulted in a decreased brain norepinephrine level [1, 36] whereas an increased brain norepinephrine concentration was found following isolation stress [24, 39], cold exposure [15], heat [23] or muricide [29]. Thus, it is apparent that changes in central catecholamine level may also vary depending on the stimulus applied.

In this investigation, repeated exposure to air blasts resulted still in a significant increase in hypothalamic histamine concentration although no alterations in the other examined brain regions were noted. The plasma corticosterone level was raised to ten-fold that of control values. This is in contrast to the repeated "platform stress" where adaptation to both hypothalamic histamine elevation and to plasma corticosterone alteration was observed [22]. It is of interest that hypothalamic norepinephrine depletion was observed following the first exposure to immobilization stress but no reduction was seen after repeated exposures [18]. It seems possible that a certain adaptation may ensue to one type of

stressful stimulation but does not occur when another type of stress is applied. This is consistent with the reported elimination of footshock induced brain norepinephrine depletion in animals previously exposed to repetitive footshocks but not following repetitive swim pretreatment [42]. It was also noted that adaptation to the increase in plasma corticosterone occurred in the former group of rats but not in the latter [42].

Although, in the present studies, in most instances an increase in plasma corticosterone was associated with an increase in hypothalamic histamine level, a strict relationship between these two events could not be demonstrated: plasma corticosterone level was increased 14-fold after 30 min exposure to air blasts while hypothalamic histamine was only slightly increased (18%). Interactions between brain histamine and corticosteroids are known to occur, however, the mechanism involved is far from being understood. In rats hypophysectomy was reported not to affect hypothalamic histamine concentration or the formation of ^{3}H-histamine from intraventricularly administered ^{3}H-histidine [37]. Adrenalectomized mice exhibited a lower histidine decarboxylase activity which resulted in a significant decrease in brain histamine level, but they still could respond to stress by a decreased ^{3}H-histamine turnover [41].

It is of interest that histamine was reported to induce a significant elevation in plasma prolactin level in rats [7] and in men while growth hormone was not affected [26]. Although plasma prolactin concentration is known to be increased following stressful situations [25,34], a possible link between stress induced alterations in brain histamine level and the reported plasma prolactin changes remains to be elucidated. It was suggested that prolactin secretion stimulated by novel environmental stress may be mediated by noradrenergic neurones ascending in the medial forebrain bundle [9]. Curiously enough an ascending histaminergic pathway was also suggested in this brain region [10].

Brain histamine seems to be involved in thermoregulation [14], water intake [20] and behaviour [5,11]. Present report is consistent with our previous observations [22] and suggest again a role of brain histamine in the response to stress.

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