Glucocorticoid Attenuates Increases in Rat Brain Noradrenaline Turnover Induced by Intense Stress

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Summary: The effects of altered pituitary-adrenocortical function on contents of noradrenaline (NA) and its principal metabolite, 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO₄) in brain regions of the rat exposed to acute intense stress (electric tail shock under conditions of immobilization) were investigated. Bilateral adrenalectomy significantly potentiated the stress-induced decreases in NA contents in the hypothalamus and thalamus, and the increases in MHPG-SO₄ levels in the hypothalamus, amygdala, and thalamus. Supplementary administration of corticosterone attenuated both of these decreases of the amine and the increases of the metabolite. The results suggest that glucocorticoid secreted via the pituitary-adrenocortical axis has an inhibitory effect in part on the increased turnover of NA in some brain regions induced by acute intense stress.

Key words: noradrenaline — 3-methoxy-4-hydroxyphenylethyleneglycol sulfate — adrenalectomy — corticosterone — acute intense stress

Introduction

During acute intense stress, brain noradrenaline (NA) depletion has been shown to reflect strongly enhanced NA release which exceeds the amine synthesis (Bliss et al 1968; Stone 1973; Stolk et al. 1974). In our previous study (Nakagawa et al. 1981), NA contents in rat brain regions decreased and were then maintained at the decreased levels during 180 min of electric tail shock under immobilization, conditions which were considered to be extremely intense. Levels of 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO₄), a principal metabolite of NA (Schanberg et al. 1968) which reflects release of the amine in the rat brain (Korf et al. 1973; Sugden and Eccleston, 1971), increased

progressively during the same period of stress.

The glucocorticoids, secreted via the pituitary-adrenocortical axis, plays an important role in an organism's mechanism for coping with environmental stress (Selye 1950). Some evidence indicates that variations in this system have significant effects on increased brain NA turnover of rats exposed to acute stress (Hedge et al. 1979; Vellucci 1977). The present experiment was undertaken to examine the possible role of glucocorticoids in the characteristic response of brain noradrenergic neurons to acute intense stress. We examined the effect of abolishing glucocorticoid secretion via adrenalectomy on the stress-induced changes in contents of NA and MHPG-SO₄, and compared these find-

ing with those obtained both with and without supplemental administration of corticosterone. The hypothalamus, amygdala, and thalamus were the brain regions chosen in the present study, because noradrenergic neurons in these three regions show rapid and marked response to the above stress or immobilization alone (Tanaka et al. 1982).

Methods

Animals

Male Wistar rats weighing 250–300 g were used in the experiment. Four rats were housed in a plastic cage (210×320×135 mm) at constant temperature ($24\pm1^{\circ}\text{C}$) under a 12 hr light/dark cycle (on 0700, off 1900). These animals were divided into three groups: sham-operated; adrenalectomized; and adrenalectomized plus corticosterone treated. These three groups were subsequently divided into two subgroups: non-stressed; and stressed. Each subgroup consisted of 14 rats. Bilateral adrenalectomy was performed on 84 rats by the dorsal approach under ether anesthesia. 42 rats received sham-operation. Adrenalectomized rats were given postoperatively 0.9 % NaCl solution and standard diet *ad lib*. Sham-operated animals were given food and water *ad lib*.

Experimental Procedure

The experiment was performed on the 10th day after the operation. Half of the adrenalectomized rats were subcutaneously injected with 100 mg/kg of corticosterone (Sigma Chemical Co.) suspended in olive oil. The vehicle (olive oil) was subcutaneously injected in a volume of 0.2 ml/100 g weight into sham-operated and the remaining adrenalectomized animals. Two hours after the injection, each rat was immobilized with a wire mesh (3×3 mm) and received an electric shock through an electrode apparatus attached to the tail for either 60 or 120 min. The shock was de-

livered using a constant voltage stimulator in square-wave pulses (5 Hz) for 5 sec every 30 sec at 15 V intensity (AC, about 2–3 mA). The rats were sacrificed by decapitation in both the non-stressed and stressed groups immediately before and immediately following the stress, respectively.

Tissue Preparation and Biochemical Determination

The brain was removed and rapidly dissected into discrete regions on an ice plate according to the method of Gispen et al. (1972). The brain regions dissected were: the hypothalamus, amygdala, and thalamus. Contents of NA and MHPG-SO₄ in each brain region (two sample pooled) were determined by the method of Kohno et al. (1979). Trunk blood was collected and plasma corticosterone levels were determined based on the method of van der Vies (1961). All samples were stored at -45°C until assayed.

Statistical Evaluation

All statistical comparisons were made using analysis of variance (at least $P<0.05$) with two factors, *i. e.* treatment (sham-operation, adrenalectomy, adrenalectomy plus corticosterone treatment) and time (0, 60, 120 min), and subsequently Turkey post-hoc pairwise comparisons ($\alpha=0.05$), unless otherwise noted.

Results

Plasma corticosterone levels of sham-operated rats were markedly elevated by the combined stress of shock plus immobilization (the mean values $\pm$ S.E.M. were: 41.0 ± 1.0 $\mu\text{g/dl}$ at 60 min and 41.8 ± 1.4 $\mu\text{g/dl}$ at 120 min) as compared with non-stressed rats (10.5 ± 1.6 $\mu\text{g/dl}$). Corticosterone levels were not detected in either non-stressed or stressed adrenalectomized rats. When adrenalectomized rats were

Fig. 1. Effect of altered pituitary-adrenocortical function on contents of NA and MHPG-SO₄ in brain regions of rats exposed to acute intense stress.

Open columns are sham-operated groups, solid column adrenalectomized groups, and hatched columns adrenalectomized plus corticosterone-treated groups. Each values indicates the mean $\pm$ S.E.M. (N=6-7). Statistically significant differences between the groups are shown by the connecting bars ($P < 0.05$).

treated with corticosterone (100 mg/kg), plasma corticosterone levels were much higher than those of sham-operated animals before the stress ($94 \pm 10.4 \mu\text{g/dl}$) and during the stress ($88.4 \pm 3.8 \mu\text{g/dl}$ at 60 min and $96.8 \pm 5.0 \mu\text{g/dl}$ at 120 min).

Analysis of variance showed significant treatment effects on NA contents in the hypothalamus; $F(2, 52) = 5.52$ $P < 0.01$ and thalamus; $F(2, 52) = 6.05$ $P < 0.01$ and on MHPG-SO₄ levels in the hypothalamus; $F(2, 52) = 23.54$ $P < 0.01$, amygdala; $F(2,$

53)=5.10 $P<0.01$, and thalamus; $F(2, 52)=10.95$ $P<0.01$.

As shown in Fig. 1 (0 min), adrenalectomy by itself significantly decreased NA content and increased MHPG-SO₄ level in the hypothalamus, but not in the amygdala and thalamus. In the hypothalamus and thalamus, the stress caused significantly greater decreases in NA contents in adrenalectomized rats than those observed in sham-operated animals (60 and 120 min). Stress-induced increases in MHPG-SO₄ levels in adrenalectomized rats were significantly greater than those in sham-operated animals in the hypothalamus, amygdala, and thalamus (60 and 120 min). The stress caused significantly smaller reductions of NA contents in the hypothalamus (120 min) and thalamus (60 and 120 min) in adrenalectomized rats pretreated with corticosterone as compared with those without such treatment. Increases in MHPG-SO₄ levels caused by the stress in adrenalectomized rats were significantly attenuated by the supplemental administration of corticosterone in the hypothalamus and amygdala (60 and 120 min), and thalamus (120 min), however, these increases in MHPG-SO₄ levels were still significantly greater than those in sham-operated animals.

Discussion

Adrenalectomy, which abolished glucocorticoid secretion, potentiated increases in MHPG-SO₄ levels in the hypothalamus, amygdala, and thalamus induced by acute intense stress consisting of electric tail shock under immobilization. Pretreatment with corticosterone, which replenished the glucocorticoids in adrenalectomized rats, attenuated the accelerated increases in the metabolite levels in the three brain regions examined. The stress-induced decreases in NA contents in the hypothalamus and thalamus were also potentiated by adrenalectomy and the accelerated NA reductions were

reversed by supplemental administration of corticosterone. These findings suggest that corticosterone secreted via the pituitary-adrenocortical axis has an inhibitory role in part on enhanced NA turnover induced by stress in some brain regions of the rat. The present results are consistent with the finding of Endrczi et al. (1976) who demonstrated that stress-induced disappearances of tritiated NA in the hypothalamus and hippocampus of adrenalectomized rats were abolished by hydrocortisone implantation into the tubero-infundibular area.

Ruhmann-Wennhold and Nelson (1977) have indicated that markedly elevated plasma ACTH levels in adrenalectomized and stressed rats can be completely suppressed by dexamethasone treatment. ACTH has been shown to facilitate NA turnover in the intact rat brain (Biscardi and Naller, 1972; Javoy et al. 1968; Shen and Ganong, 1976). Together with these findings, it appears that the attenuating effect of corticosterone on stress-induced increases in brain NA turnover accelerated by adrenalectomy may result from decreased ACTH secretion via a negative feed back system. On the other hand, the suggestion that ACTH might provoke emotionality in the rat (DiGiusto et al. 1971; Weiss et al. 1969) raises the possibility that "anxious" rats (produced by increased circulation of ACTH under conditions of adrenalectomy) respond strongly to stress so that their NA turnover is markedly enhanced. Corticosterone treatment reduces ACTH secretion and so may relieve such a strong response in adrenalectomized animals. Interestingly, the present results showed that corticosterone possessed the attenuating effect on noradrenergic activity in the hypothalamus which is a structure having important role in pituitary-adrenocortical function and emotions, as well as in the amygdala which has close anatomical connection with the hypothalamus (Henke, 1979). Moreover, we obtained the same effect of corticosterone on the thalamus, which regulates

arousal level through relaying afferent sensory inputs.

Stress enhances amine synthesis in brain noradrenergic neurons, especially as Thierry et al. (1971) have suggested, via a process whereby newly synthesized NA is increased and selectively utilized in the rat brain during acute stress. Generally, NA concentration in the nerve terminals are regulated by balancing release and synthesis of the amine. We proposed the existence of a mechanism which supplies this available NA to protect NA stores from exhaustive depletion in the rat brain caused by continuously enhanced release of NA induced by acute intense stress (Nakagawa et al. 1981). Glucocorticoids may contribute in part to this supplementary regulation system. This idea is supported by the present finding that in the thalamus, corticosterone treatment reversed NA depletion potentiated by adrenalectomy without affecting MHPG-SO₄ level after 60 min of the stress.

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