Bilateral Adrenalectomy Does Not Alter Stress-Induced Increases in Noradrenaline Turnover in Rat Brain Regions

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Received for publication December 14, 1982

Summary: Effects of adrenalectomy on increases in noradrenaline (NA) turnover induced by a one-hour immobilization stress were examined by measuring the levels of NA and its major metabolite, 3-methoxy-4-hydroxyphenylethyneglycol sulfate (MHPG-SO₄) in specific brain regions of the rat. Bilateral adrenalectomy alone does not affect basal levels of NA and MHPG-SO₄ in the brain regions examined with the exception of an increase in MHPG-SO₄ levels in the hypothalamus and a decrease in NA levels in the cerebral cortex. In sham-operated rats, immobilization stress caused significant increases in MHPG-SO₄ levels in five brain regions that were examined, i.e., the hypothalamus, amygdala, hippocampus, cerebral cortex and pons plus medulla oblongata, and significant decreases in NA levels in the hypothalamus and amygdala. These changes in NA and MHPG-SO₄ levels induced by stress were not affected by bilateral adrenalectomy. These results suggest that corticosterone is not a causative factor in enhancing brain NA turnover during immobilization stress.

Key words: adrenalectomy — immobilization stress — noradrenaline — MHPG- SO₄ — noradrenaline turnover — rat brain regions

Introduction

Various stressful stimuli increase noradrenaline (NA) turnover in many regions of the rat brain (Nakagawa et al. 1981 a; Tanaka et al. 1982 a; 1982 b; Tsuda et al. 1982). Stress-induced increases in NA turnover in the hypothalamus, thalamus and amygdala may be regulated by endogenous opioids and are modified by administration of a potent opiate, morphine (Tanaka et al. 1981 a; 1981 b; 1982 b).

A marked elevation of plasma corticosterone levels occurs during stressful situations in rats. The relationship between the markedly elevated corticosterone levels during stress and the enhancement of NA turnover during stress has not been resolved. Rats were exposed to electric tail shock while immobilized. This was a very stressful situation. Nakagawa (1981 b) found that bilateral adrenalectomy significantly potentiated this intense-stress-induced decrease in NA content and also potentiated the increase in the levels of the major metabolite of NA, 3-methoxy-4-hydroxyphenylethyneglycol sulfate (MHPG- SO₄) in some regions of the brain. Moreover, these potentiated decreases of the amine and the increases of the metabolite by adrenalectomy were attenuated by administration of corticosterone (Naka-
In the present study, the effects of adrenalectomy on increases in brain NA turnover of rats induced by immobilization were investigated. Immobilization stress is less intense and is more commonly used in stress studies than immobilization with tail-shock.

**Methods**

Thirty-two male Wistar rats weighing 170-190 g were housed 4 to a cage (265×425×150 mm standard plastic cage containing wood shavings) at a constant room temperature (24±1°C) and humidity (50±10%) under a 12-hour (light time; 0700-1900) light/dark cycle. Food and water were provided *ad libitum*.

Bilateral adrenalectomy was performed on 16 rats by the dorsal approach under ether anesthesia. The remaining sixteen rats received a sham-operation in which a dorsal incision was made under ether anesthesia and the adrenal glands were exposed, but not removed. After the operation, the adrenalectomized animals were kept on a standard diet with 0.9% saline. Sham-operated rats were given the standard diet and water.

Each experiment was performed on the eighth day after the operation. Half of the adrenalectomized rats and half of the sham-operated rats were exposed to immobilization-stress for 1 hour by enclosing the animals in a flexible wire mesh (3×3 mm), as described previously (Tanaka et al. 1982 a; 1982 b). The remaining half of the adrenalectomized and sham-operated rats served as controls.

Immediately after the 1-hour stress procedure, the rats were sacrificed by decapitation. The brain was rapidly removed and dissected into discrete brain regions according to the method of Gispen et al. (1972) and frozen on solid CO$_2$. The brain regions dissected were; hypothalamus, amygdala, hippocampus, cerebral cortex and pons plus medulla oblongata (pons+med. obl.). Following decapitation, blood was collected from the cervical vessels in heparin-containing tubes. Separated plasma and brain tissues were stored at -45°C until assayed. NA and MHPG-SO$_4$ levels in the brain regions were determined simultaneously by a fluorometric method (Kohno et al. 1979). Plasma corticosterone levels were determined fluorometrically by the method of van der Vies (1961) with slight modifications.

The experiment was carried out between 1000 and 1400 hours. No diurnal variations of either NA or MHPG-SO$_4$ contents have been observed during this period (Kohno et al. 1980). The statistical analysis involved a two-tailed Student’s t-test.

**Results**

Plasma corticosterone levels in sham-operated rats were significantly elevated by the 1-hour immobilization stress. The mean±S.E.M. of eight control unstressed rats was 12.5±1.94 μg/dl and the mean±S.E.M. of eight stressed rats was 32.7±1.96 μg/dl (P<0.001).

No corticosterone levels were detected in adrenalectomized rats in either the control or the stressed groups. Bilateral adrenalectomy by itself significantly decreased the NA levels in the cerebral cortex and increased the MHPG-SO$_4$ levels in the hypothalamus (compared to sham-operated rats), however, neither the NA levels nor the MHPG-SO$_4$ levels were affected by adrenalectomy in other regions of the brain (Figs. 1-2).

One-hour of immobilization stress significantly increased the MHPG-SO$_4$ levels in all brain regions examined in both sham-operated and adrenalectomized rats. There were no significant differences between sham-operated and adrenalectomized rats (Fig. 2). The NA levels in the hypo-
thalamus and amygdala were significantly reduced by stress in both sham-operated and adrenalectomized rats (Fig. 2). The NA levels in the hippocampus were decreased by stress only in the adrenalectomized rats. Immobilization stress caused no significant changes in the NA levels in the cerebral cortex and pons + med. obl. in either sham-operated or adrenalectomized rats. When the rats were exposed to stress, the NA levels in the cerebral cortex of sham-operated rats were significantly greater than those in adrenalectomized rats.

Fig. 1. Effects of immobilization stress on noradrenaline (NA) levels in the five brain regions in sham-operated (SHAM) and bilateral adrenalectomized (ADX) rats. The stress was immobilization for one hour. Each value represents the mean ± S.E.M. of eight rats. The asterisk above a column indicates a statistically significant difference from the respective controls. The NA levels in the cerebral cortex of SHAM-CONTROL and SHAM-STRESS animals were significantly greater than those in ADX-CONTROL and ADX-STRESS animals, respectively (P<0.05, P<0.01).
Fig. 2. Effects of immobilization stress on the levels of the major metabolite of NA, 3-methoxy-4-hydroxyphenylethleneglycol sulfate (MHPG-\textsubscript{SO}_4) in the five brain regions of sham-operated (SHAM) and bilaterally adrenalectomized (ADX) rats. Each value represents the mean±S.E.M. of eight rats. The asterisk above a column indicates a statistically significant difference from the respective controls. The MHPG-\textsubscript{SO}_4 levels in the hypothalamus in ADX-CONTROL animals were significantly greater than those in SHAM-CONTROL animals (P<0.01).

Discussion

There have been conflicting reports on the effects of adrenalectomy on brain NA metabolism in rats. In many studies, adrenalectomy has been reported to increase NA turnover in the brain (Javoy et al. 1968; Leonard, 1974; Shen and Ganong, 1976a; Endrőczi et al. 1976). It has also been reported that the activities of enzymes involved in the synthesis and degradation of catecholamines are changed by adrenalectomy. Adrenalectomy increases MAO activity in the hypothalamus (Parvez and Parvez, 1973) and decreases the activity of both tyrosine hydroxylase in the median eminence (Kizer et al. 1974) and dopamine-\textbeta-hydroxylase in the hypothalamus and brain stem (Shen and Ganong, 1976b).

The previous reports suggest that corticosterone may influence neuronal transmission mediated by NA in the central nervous system. The present study demonstrates that in most regions of the brain the levels of neither NA nor its principal metabolite, MHPG-\textsubscript{SO}_4, are affected by bilateral adrenalectomy, which abolishes corticosterone secretion. The exceptions are an increase in MHPG-\textsubscript{SO}_4 levels in the hypothalamus and a decrease
in NA levels in the cerebral cortex. Since the levels of MHPG-SO$_4$ in the non-stressed state are indicative of NA turnover (Kohno et al. 1981), NA turnover in the hypothalamus appears to be increased by adrenalectomy. The decreased NA levels in the cerebral cortex may result from a decreased activity of the synthesizing enzyme, tyrosine hydroxylase. These findings are in basic agreement with previous results, however, it is questionable how specific these changes are. Glucocorticoid and also mineralocorticoid would be eliminated by adrenalectomy. Supplemental corticosterone was not administered in the present study. The present study demonstrates that bilateral adrenalectomy produces no marked effects on the levels of either NA or MHPG-SO$_4$ in the brain of non-stressed rats for at least 7 days after the operation.

Consistent with our previous reports (Tanaka et al. 1981a; 1981b; 1982a; 1982b), a one-hour immobilization stress caused significant increases in MHPG-SO$_4$ levels in all five brain regions examined in sham-operated rats. There were also significant reductions of NA levels in the hypothalamus and amygdala. This suggests that NA turnover in these five brain regions is increased by stress. In adrenalectomized rats, immobilization stress significantly increased the levels of MHPG-SO$_4$ in the five regions and decreased the levels of NA in the hypothalamus, amygdala and hippocampus. Thus, adrenalectomy did not alter the response to stress. This finding indicates that stress-induced increases in NA turnover in the sham-operated animals are not due to increases in plasma corticosterone. This conclusion is not consistent with the findings of Nakagawa (1981b). The intense stress, electrical tail shock under immobilization, caused significantly greater decreases in NA levels in the hypothalamus, amygdala and thalamus in adrenalectomized rats than in sham-operated rats. The enhanced increases in MHPG-SO$_4$ levels and decreases in NA levels in adrenalectomized rats were attenuated by administration of corticosterone (Nakagawa, 1981b).

The discrepancy in the effects of bilateral adrenalectomy on increases in NA turnover induced by the two different stresses may be due to the different intensities of the stresses employed. Under intense stress, increased corticosterone may attenuate stress-induced increases in NA turnover in specific brain regions. Under less intense stress, such as immobilization stress, corticosterone does increase but it either does not alter NA turnover or the change in NA turnover is too small to detect with biochemical methods. These contradictions can be explained if stress-induced increases in NA turnover that are mediated by increased corticosterone depend on the intensity of the stress employed.

In conclusion, the increases in plasma corticosterone levels are not the causative factor which increases NA turnover in specific brain regions during immobilization stress. Moreover, corticosterone does not mediate the increases in NA turnover induced by immobilization stress.

Acknowledgements: We are grateful to Ms. Shigeko Takeda for her skillful technical assistance and to Ms. Miyo Kato for preparing the figures. Gratitude is also due to Nippon Roche K.K. for the generous supply of MHPG-SO$_4$.

References

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