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Central elevation of phenylethanolamine *N*-methyltransferase activity following stress

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Phenylethanolamine *N*-methyltransferase (PNMT, E.C.2.1.1.28) is the terminal enzyme in the synthesis of adrenaline in the adrenal medulla[1], other chromaffin tissues, and in the mammalian brain[17]. Changes in the level of adrenal and hypothalamic PNMT activity have been correlated with changes in the tissue content of adrenaline[13,15,18,20]. It is well established that PNMT requires glucocorticoids for maximal activity. The synthetic glucocorticoid, dexamethasone, has been shown to induce adrenal PNMT in hypophysectomized rats[19] and to stimulate the enzyme in extra-adrenal chromaffin tissue of neonatal rats[2,6] and brain of adult rats[13]. Induction occurs over a period of days and is presumed to be due to an increase in the rates of enzyme synthesis[5,20]. The dose of dexamethasone employed to produce PNMT stimulation has been very high, generally 0.5–1.0 mg/kg.

The physiological role of glucocorticoids in the regulation of PNMT in the intact animal is less clear. Adrenal PNMT activity may be regulated by ACTH (indirectly acting through adrenal glucocorticoid synthesis), by circulating glucocorticoids directly, and also by neuronal activity via the splanchnic nerves in different strains of mice[5] and rats[11]. Short-term treatment with dexamethasone has been reported to be without effect on adrenal PNMT in the intact animal[19,20], although the activity of the adrenal enzyme is increased 40 days after unilateral adrenalectomy[3] and the brain enzyme after 13 days of dexamethasone treatment[13]. The half-life of adrenal PNMT, estimated without blockade of protein synthesis, is reported to be quite long (6 days)[4]. Based on these considerations, Ciaranello and Black[4] have suggested that PNMT finds its biological role only in chronic stress situations. Nothing is known regarding the effect of stress on the activity of PNMT in brain tissue.

The neuroanatomical positions of the adrenergic cell bodies in the rostral medulla oblongata of the brain stem[10] is suggestive of a relationship to stress. The ventral adrenergic cell group (C_1) coincides in part with the vasomotor center[9] and is thought to project to the spinal cord, the hypothalamus, and other forebrain regions known to mediate aspects of the stress response[10]. The dorsal cell group (C_2) is in part coincident with the cardioinhibitory center (the dorsal motor nucleus of the vagus)[9] which receives input from baroreceptors.

TABLE I

Effect of 30 minute shaker stress on brain and adrenal PNMT activity

The PNMT assay of Pendleton was modified to employ [^{3}H]methyl-*S*-adenosyl-l-methionine (New England Nuclear Corp), specific activity 10.2 and 10.5 Ci/mmole as the methyl donor together with unlabeled carrier to yield a final incubation molarity of 3×10^{-5} *M*. Triplicate determinations were performed on all samples, using only organic extraction of the labeled product. Proteins were measured by the method of Lowry[18]. Plasma corticosterone was determined by radioimmunoassay. Values given are the mean ± S.E.M. of 16 rats in each group.

	Brain Stem PNMT Activity pmoles/mg protein/h	*Adrenal PNMT activity pmoles/adrenal pair/h*	*Plasma corticosterone μg/100 ml*
Control	37.0 ± 3.0	5090 ± 319	2.1 ± 0.4
30 Min Stress	47.7 ± 2.5*	4949 ± 290	39.9 ± 2.3

* Statistically significant $P < 0.01$ against control values (Student's *t* test).

We therefore investigated the possibility that acute stress, with its attendant rise in plasma corticosterone, could stimulate brain PNMT activity. Sprague–Dawley rats (250–400 g) were injected with saline, a standard procedure in our laboratory to allow comparison between experiments, and 30 min later were either subjected to horizontal shaker stress for 30 min or allowed to remain undisturbed in individual cages. Rats were killed by decapitation, and trunk blood collected for plasma corticosterone determination. Brains and adrenals were removed immediately and placed on a chilled glass platform. A rectangular punch device, 5 mm × 8 mm, was used to standardize a dissection of the medulla oblongata and pons which includes the PNMT containing C_1 and C_2 nuclei. Adrenals and dissected brain stems were then frozen in liquid nitrogen prior to assay. The assay used for PNMT activity was that of Pendleton with modifications[14].

The short term shaker stress produced a 29% increase in brain PNMT ($P < 0.01$) without altering adrenal PNMT activity (Table I). The stress employed was sufficiently potent to produce an 18-fold rise in plasma corticosterone. These data indicate that PNMT in brain tissue, in marked contrast to that of the adrenal medulla, is capable of response to stress. Our results are in agreement with a previous report showing no acute change in adrenal PNMT activity in response to stress[11].

The stress-dependent increase in brain PNMT activity is very rapid, occurring within 30 min; maximal stimulation of enzyme activity in the adrenals of hypophysectomized animals treated with dexamethasone requires between 3 and 7 days[5,20]. The elevation in central nervous system PNMT activity therefore may be the result of a mechanism other than that implicated in the adrenal system. The latter is presumed to be enzyme induction based on its blockade by protein synthesis inhibitors and other inferential evidence[5,20]. Stress could produce a direct activation of brain PNMT, either through a decrease in ionic strength in the immediate environment of the enzyme (physiological concentrations of NaCl, KCl and other salts have been shown to competitively inhibit PNMT activity)[7] or by a decrease in S-adenosylhomocysteine

which is also known to inhibit enzyme activity[8]. No direct evidence exists for either of these hypotheses.

Regardless of mechanism, the increase in brain PNMT activity which accompanies acute stress constitutes the first evidence that the synthesis of central nervous system adrenaline is augmented during stress. The data strongly suggest that the adrenergic system in the brain stem may be involved in the physiological regulation of the stress response. A recent report has demonstrated elevated PNMT activity in the C_1 and C_2 regions of the brain stem in genetic and experimental hypertensive rats[16]. Our data indicate that the rise seen in spontaneous hypertension may be a more general phenomenon, occurring as part of the normal stress response.

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