Regulatory aspects of S-adenosylmethionine in the periphery and CNS

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INTRODUCTION

S-adenosylmethionine (SAM) assumes an active role as a regulator in many biological systems in addition to its role as a cofactor in transmethylation reactions. Our interest in this nucleoside arose as a consequence of studies on the *in vivo* regulation of the biogenic amine enzymes, phenylethanolamine N-methyltransferase (PNMT) and hydroxyindoleamine O-methyltransferase (HIOMT).

Earlier studies on PNMT, the final enzyme in the epinephrine pathway, showed that the intracellular degradation of this enzyme was controlled via hormonal stimuli (Ciaranello, 1978). Hypophysectomy decreases enzyme activity while glucocorticoid administration restores PNMT levels. Similar studies on the *in vivo* regulation of HIOMT (Sandrock *et al.*, 1980), the terminal enzyme in melatonin synthesis, revealed that it was regulated in a similar fashion to its catecholamine counterpart, PNMT.

In vitro degradation studies using the proteolytic enzyme, trypsin, showed that both PNMT and HIOMT were protected against proteolysis by the presence of the enzymatic cofactor, SAM (Ciaranello *et al.*, 1978). This suggested the possibility that glucocorticoids might regulate *in vivo* SAM levels in the adrenal and pineal, the primary tissue sources of these enzymes.

E. Usdin et al., *Biochemistry of S-Adenosylmethionine and Related Compounds*

Glucocorticoid Regulation of Adrenal and Pineal SAM

The role of SAM as an endogenous inhibitor of PNMT degradation was supported by three findings. First, an "endogenous stabilizing factor" was isolated from tissue extracts which protected the enzyme against thermal and tryptic degradation, and which possessed physical, spectral and chromatographic properties similar to SAM. Second, the presence of SAM during the tryptic degradation of PNMT provided a four-fold protection against proteolysis (Berenbeim *et al.*, 1979). Third, administration of SAM to hypophysectomized animals restored *in vivo* PNMT levels as effectively as either dexamethasone or ACTH administration.

If SAM mediates the glucocorticoid regulated degradation of PNMT, its levels should change concertedly with glucocorticoid induced changes in PNMT activity. Using a radioisotopic dilution assay developed in our laboratory, which measures SAM in the absence of its metabolite, SAH, we followed changes in adrenal SAM concentrations as a consequence of hypophysectomy and glucocorticoid replacement (Table 1). SAM concentrations in adrenal tissue decreases following hypophysectomy as does PNMT activity. Dexamethasone administration to hypophysectomized animals restores both SAM and PNMT activity simultaneously. Thus, glucocorticoids appear to regulate *in vivo* concentrations of SAM, which, in turn, regulate enzyme degradation.

Table 1. Effects of Glucocorticoids on PNMT Activity and SAM Concentrations in Rat Adrenal

	PNMT Activity Units/Pair	[SAM] µg/Pair
Control	1.60 ± 0.01	0.49 ± 0.08
Hypophysectomized	0.45 ± 0.01[a]	0.23 ± 0.05[c]
Hypophysectomized + Dexamethasone	0.87 ± 0.01[b]	0.69 ± 0.09[d]

a) $p = 10^{-4}$, significantly different from control
b) $p = 10^{-9}$, significantly different from hypophysectomized
c) $p = 0.02$, significantly different from control at 2 percent
d) $p = 0.12$, not significantly different from control
e) $p = 10^{-3}$, significantly different from hypophysectomized

Table 2. Effects of Glucocorticoids on HIOMT Activity and SAM Concentration in Rat Pineal

	HIOMT ACTIVITY Units/Pair	[SAM] µg/Gland
Control	79.9 ± 1.2	0.35 ± 0.03
Hypophysectomized	27.0 ∓ 5.6[a]	0.15 ∓ 0.01[c]
Hypophysectomized + Dexamethasone	59.8 ∓ 5.6[b]	0.19 ∓ 0.01[d]

a, c) $p = 10^{-4}$, significantly different from control
b) $p = 0.015$, significantly different from hypophysectomized
d) $p = 0.03$, significantly different from hypophysectomized

Since SAM is also utilized as a cofactor by the indoleamine methyltransferase, HIOMT, we investigated the possibility that this enzyme was similarly controlled by SAM and glucocorticoids. As in the case of PNMT, hypophysectomy caused a simultaneous reduction in HIOMT activity and SAM concentration. Dexamethasone administration to hypophysectomized animals reverses these effects (Table 2). Thus, adrenal cortical hormones play an important role in the maintenance of SAM and HIOMT and SAM and PNMT in the pineal and adrenal, respectively.

Peripheral and Central Glucocorticoid Regulation of SAM

The observed glucocorticoid modulation of SAM concentrations in the pineal and adrenal indicated that these hormones might be essential in other SAM-dependent methylations. Although a role for glucocorticoids has not been demonstrated in most cases, their importance in maintaining SAM levels in the adrenal and pineal, suggested that we should also study the role of glucocorticoids in other SAM-containing tissues, such as the liver and the brain.

In these studies, the brain regions chosen were those which actively incorporate and bind glucocorticoids, presumably sites of glucocorticoid receptors. Some caution must be observed as the regional assignment of glucocorticoid receptors depends on the specific glucocorticoid radioligand utilized in the studies (McEwen, 1977). For instance, in rat, tritiated dexamethasone predominantly labels corticoid receptors in the hypothalamus, amygdala, septa, hippocampus, and cortex while tritiated corticosterone primarily labels glucocorticoid receptors in hippocampus, septa, and amygdala.

Figure 1 shows the results of these studies on corticoid influences in brain and peripheral tissue in rats. Although glucocorticoids appear to affect SAM levels in some cases, glucocorticoids are not the sole regulators of this nucleoside. Three patterns emerge from the data. In striatum and midbrain, hypophysectomy reduces SAM content while dexamethasone administration restores the nucleoside toward normal values. This is consistent with the pattern observed in adrenal and pineal glands. In thalamus, hypothalamus, hippocampus and cerebellum, hypophysectomy increases SAM concentrations but no further changes are effected by dexamethasone administration. Finally, neither hypophysectomy nor dexamethasone has an effect on SAM content in the cortex, septum, pons-medulla, and liver.

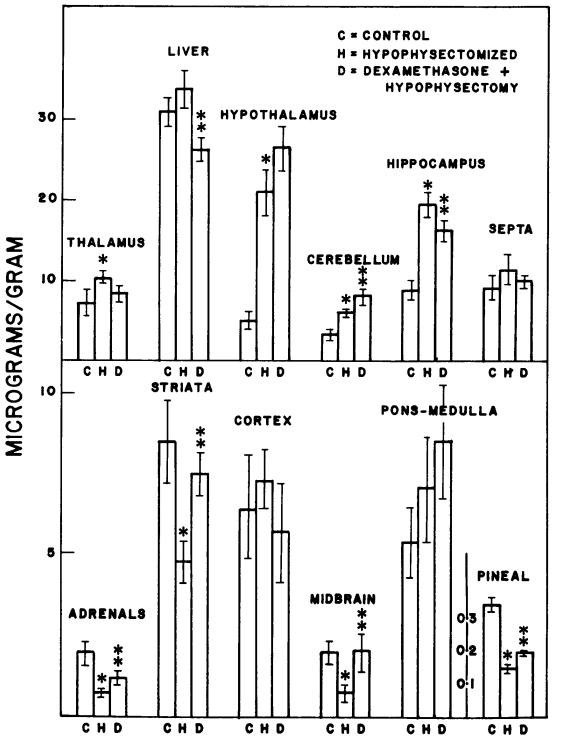

Figure 1 Regional Distribution of S-adenosylmethionine in Brain and Peripheral Tissue of Control, Hypophysectomized, and Dexamethasone Treated Rats.

The preceding results suggest that although glucocorticoids may be important in regulating SAM concentrations in some peripheral tissues and brain regions, they are not the sole regulatory factors for SAM in vivo. Other endocrine factors may be important. In tissues where glucocorticoids are ineffective in reversing the SAM changes produced by hypophysectomy, other pituitary factors, besides ACTH, may be involved in SAM regulation in these tissues. Many of the pituitary hormones are distributed both centrally and peripherally in regions beyond their target organ realm and non-target oriented functions, in addition to target oriented functions, are well established. In contrast, in those tissues where hypophysectomy and glucocorticoids are ineffective in altering SAM concentrations, the pituitary and its hormones are probably not responsible for SAM regulation. However, hypothalamic releasing hormones, which also have biological activity apart from their induction of pituitary hormones, may be involved. In addition, we must not exclude non-endocrine factors, particularly neuronal factors. We have some preliminary evidence that factors released from the splanchnic nerve, perhaps endogenous opiate peptides, influence adrenal SAM levels. Alternatively, a combination of hormonal and neuronal factors may be essential in SAM regulation in vivo.

Changes in SAM Metabolic Enzymes

Current studies have focused on two enzymes involved in SAM metabolism, methionine adenosyltransferase (MAT) and S-adenosylhomocysteine hydrolase (SAHase). MAT converts methionine and ATP in the presence of Mg^{+2} to SAM while SAHase is responsible for the enzymatic hydrolysis of SAH to adenosine and homocysteine. Since in the adrenal glucocorticoids control PNMT degradation via SAM, we were interested in further defining the biochemical site of action of the corticoids. Do they exert their effect directly on the enzymes immediately responsible for SAM metabolism or is their effect exerted more distally?

Following the same experimental regimen utilized in the PNMT and HIOMT regulation studies, we monitored changes in MAT and SAHase in adrenals of control, hypophysectomized, and dexamethasone treated rats. MAT activity was measured according to a modification of the Chou and Lombardini procedure (1972) while SAHase activity was monitored by modification of the procedure of Glazer and Peale (1978). Table 3 shows the results of these studies. PNMT, MAT, and SAHase activity was measured simultaneously for each animal. MAT activity changes concommitantly with glucocorticoid and SAM induced alterations in PNMT

activity. Hypophysectomy leads to a reduction in the activity of both enzymes while glucocorticoid replacement restores enzyme activity. In contrast, hypophysectomy decreases SAHase activity, but dexamethasone does not restore the enzyme. Thus, glucocorticoids appear to control SAM concentrations by altering levels of MAT activity.

Table 3. Effect of Hypophysectomy and Dexamethasone on PNMT, MAT and SAHase Activity

	PNMT ACTIVITY Units/Pair	MAT ACTIVITY Units/Pair	SAHase ACTIVITY Units/Pair
CONTROL	0.76 ± 0.04	0.0270 ± 0.0020	0.122 ± 0.013
HYPOX*	0.03 ± 0.01^a	0.0025 ± 0.0003^c	0.010 ± 0.002^e
HYPOX + DEX**	0.92 ± 0.22^b	0.0075 ± 0.0013^d	0.011 ± 0.002^f

a, c, e) significantly different from control at $p \leq 3 \times 10^{-8}$
b, d) significantly different from hypophysectomized at $p \leq 10^{-3}$
f) not significantly different from hypophysectomized

* Hypophysectomized
**Dexamethasone

CONCLUSION

S-adenosylmethionine participates as both a cofactor in enzymatic transmethylation reactions and as a regulator in many biological systems. Evidence of its regulatory role is illustrated in the adrenal and pineal glands. SAM protects two methyltransferases in these tissues, PNMT and HIOMT, respectively, from intracellular destruction by endogenous proteases. SAM, in turn, is glucocorticoid-regulated in these tissues. In the adrenal, glucocorticoids appear to exert their effect via regulation of MAT, the enzyme responsible for SAM synthesis. SAM also appears to be controlled by glucocorticoids in certain brain regions, such as striatum and midbrain while SAM in other brain regions is probably controlled by other hormonal and neuronal factors. Future studies may reveal these factors and the sites upon which their controls are exerted.

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