EFFECTS OF HYPOPHYSECTOMY AND DEXAMETHASONE ADMINISTRATION ON CENTRAL AND PERIPHERAL S-ADENOSYLMETHIONINE LEVELS¹

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Abstract

The effects of hypophysectomy and dexamethasone administration on S-adenosylmethionine (SAM) levels in the adrenal, liver, pineal, and various brain regions were examined to determine the central and peripheral relationships between SAM and glucocorticoids *in vivo*. A simple and sensitive radioenzymatic assay was developed to measure tissue SAM concentrations following removal of its demethylated metabolite, S-adenosylhomocysteine (SAH), whose presence precludes accurate SAM determinations.

Three patterns of SAM control emerged. In the adrenal, pineal, striatum, and midbrain, SAM levels fell after hypophysectomy and were restored by dexamethasone administration. In the thalamus, hypothalamus, hippocampus, and cerebellum, SAM levels increased after hypophysectomy and were not altered further by dexamethasone administration. In the liver, cortex, septum, and pons-medulla, SAM levels were not affected by either hypophysectomy or dexamethasone administration.

These results suggest that multiple controls regulate SAM levels *in vivo*. The control factors are both highly tissue and region specific. While glucocorticoids are an important regulatory factor of SAM in some peripheral and CNS tissues, they are not the sole regulatory factor. In CNS regions where hypophysectomy increases SAM levels but glucocorticoid administration does not reverse the effects, other hypothalamic hormones, pituitary hormones, or neural factors may be involved in SAM regulation. Likewise, in regions where neither hypophysectomy nor glucocorticoid administration affects SAM levels, hypothalamic or neural factors may be involved even though pituitary factors do not appear to be important.

S-Adenosylmethionine (SAM) serves as the methyl donor in a variety of enzymatic transmethylation reactions. While studying the regulation of adrenal medullary phenylethanolamine N-methyltransferase (PNMT), the terminal enzyme in adrenaline biosynthesis, we observed that SAM protected this catecholamine biosynthetic enzyme against *in vitro* proteolysis by trypsin. SAM appears to protect PNMT by binding to the methyltransferase and altering its tertiary conformation so that it is less susceptible to proteolytic attack (Berenbeim et al., 1979). Since glucocorticoids control *in vivo* steady state levels of this enzyme by inhibiting proteolytic degrada-

tion (Ciaranello and Black, 1971; Moore and Phillipson, 1975; Ciaranello, 1978), we proposed that SAM acts as an intracellular regulatory agent, whose levels are controlled by glucocorticoids.

Another biogenic amine enzyme, hydroxyindole O-methyltransferase (HIOMT), the pineal enzyme involved in melatonin synthesis, is regulated in a similar fashion by glucocorticoids. HIOMT levels fall after hypophysectomy and can be restored by the administration of adrenocorticotrophic hormone (ACTH), dexamethasone, or SAM. SAM modulates the *in vitro* degradation of HIOMT as well as PNMT (Sandrock et al., 1980). Therefore, glucocorticoids may regulate SAM levels in both the adrenal and pineal, the tissues in which PNMT and HIOMT are primarily located.

It became of interest to define further the role of SAM in the *in vivo* regulation of PNMT and HIOMT degradation and in the regulation of other biological systems in the CNS and periphery. To accomplish this, we first

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developed a simple, sensitive assay for SAM which distinguishes between SAM and its demethylated metabolite, S-adenosylhomocysteine (SAH). SAH is a potent inhibitor of most methyltransferases (Deguchi and Barchas, 1971). Since most SAM radioenzymatic assays utilize methyltransferases (Baldessarini and Kopin, 1966; Yu, 1978), it is essential to separate SAM from SAH. This rapid, inexpensive, single label radioenzymatic assay was utilized in the studies on the hormonal control of SAM levels in various tissues described in this report. In some tissues and brain regions, glucocorticoids appear to be essential for the maintenance of steady state SAM levels. In others, however, separate endocrine or neuronal mechanisms may be operant. These findings suggest that SAM levels in the body are controlled by multiple factors, that endocrine influences are important, but not exclusive, regulatory factors in modulating SAM levels, and that the control of SAM is more complex than previously suspected.

Materials and Methods

Preparation of Dowex 1-X8 anion exchange resin. SAM and SAH were separated on a Dowex 1-X8, 200 to 400 mesh, HCO_3^- column (Bio-Rad, Richmond, CA) by a modification of the method of Shapiro and Ehninger (1966). The resin was pre-washed extensively with 1 M hydrochloric acid and 1 M sodium hydroxide. The resin then was equilibrated and stored in 1 M NaHCO_3 . Columns of 0.5 cm^3 bed volume were prepared in a glass microcolumn, 0.7 cm in diameter (Radnoti, Monrovia, CA), fitted with a sintered glass disk and a two-way microvalve with a drain tip. Immediately before sample application, the column was re-equilibrated with 25 ml of 1 M NaHCO_3 , followed by 50 ml of distilled water.

Preparation of hydroxyindole O-methyltransferase. Hydroxyindole O-methyltransferase (HIOMT) was prepared by a modification of the procedure of Jackson and Lovenberg (1971). Frozen beef pineals were homogenized in 50 mM Tris-HCl, pH 7.4, 0.5 mM dithiothreitol (DTT) (7.6 gm/38 ml) using a motor-driven glass homogenizer. The extract was centrifuged at $37,000 \times g$ for 30 min at 0°C . The supernatant was adjusted to 37% saturation with solid ammonium sulfate at 0°C . After equilibration for 15 min, the solution was centrifuged at $10,000 \times g$ for 20 min. The supernatant was readjusted to 65% saturation with ammonium sulfate, and the equilibration and centrifugation steps were repeated. The pellet was resuspended in 50 mM Tris-HCl, pH 7.4, 0.5 mM DTT and dialyzed overnight in the cold against the same buffer. The purified enzyme was stored at -70°C until use. Under these conditions, the purified enzyme was stable for about 2 months.

Preparation of tissue. All experiments were performed on male Sprague-Dawley rats (160 to 200 gm) obtained from Zivic Miller (Allison Park, PA). Surgical operations were performed by the supplier. Hypophysectomized animals were maintained on $\frac{1}{4}$ normal plasma salts for the duration of the experiment. Following acclimation to housing conditions for 1 week, dexamethasone was administered as described in the table and figure legends. The quality administered per injection and the number of injections were determined by dose-response curves optimizing increases in PNMT and HIOMT activity.

Rats were killed by cervical dislocation. When measuring adrenal SAM levels, the glands were removed, homogenized in 0.25 ml of 10% trichloroacetic acid (TCA) in 0.05 M hydrochloric acid, and centrifuged at $3,000 \times g$ for 30 min at 0°C . The supernatant was washed three times with 0.5 ml of ether saturated with 0.05 M HCl. Residual ether was evaporated under a gentle stream of nitrogen gas at 0 to 4°C , and samples were stored overnight at 4°C . All other tissues were homogenized in 5 vol of TCA-HCl. The remaining steps were identical to those used for adrenals with appropriate adjustments for volume differences. Blanks were prepared identically in the absence of tissue.

Immediately before application to the column, a 0.2-ml aliquot of tissue extract was diluted to 1.0 ml with distilled water. Five microliters of [^3H]SAM (Amersham/Searle, Arlington Heights, IL; specific activity adjusted to 30 mCi/mmol) was added. Fifty microliters of the diluted sample was set aside for the determination of SAM recoveries. The sample then was applied to the column. The initial 0.5 ml of effluent was discarded, and the subsequent effluent was collected. The column then was washed with 10 ml of distilled water. The first 1.5 ml of wash was collected and combined with the sample effluent. One milliliter of the combined column material was assayed for SAM content as described below. Fifty microliters of the same was counted for radioactive SAM. Recovery was estimated by comparing the counts recovered from the column with those applied. All radioactive samples were counted in 10 ml of Betaphase liquid scintillant (Westchem, San Diego).

Assay for SAM. SAM was measured using a modification of the procedure of Baldessarini and Kopin (1966); the most significant modifications were the column step separating SAM and SAH and the use of a single radioisotopic label. The assay was a radioenzymatic isotope dilution procedure in which the presence of unlabeled SAM reduced the formation of radioactive melatonin. A standard curve was established for each set of unknown tissues using unlabeled SAM purified by a modification of the method of Deguchi and Barchas (1971). Standards of 0.4, 0.8, 1.2, 1.6, and 2.0 nmol of SAM were prepared in duplicate in 1.0 ml of distilled water. To standards and tissue samples were added 25 μl of [^3H]SAM, (specific activity adjusted to 540 mCi/mmol) and 10 μl of 50 mM DTT in 50 mM Tris-HCl, pH 7.4. The reaction was begun by the addition of a solution containing 9.0 mmol of Tris-HCl, pH 8.8, 50 μg of N-acetylserotonin, and 25 μl of hydroxyindole O-methyltransferase (specific activity, 0.23 unit/ml) in a final volume of 200 μl . After incubation for 20 min at 37°C , the reaction was terminated by the addition of 0.5 ml of 0.5 M sodium borate, pH 10. [^3H]Melatonin was extracted into 5 ml of (97:3) toluene:isoamyl alcohol; 3 ml of the organic phase was counted in 10 ml of Betamax (Westchem, San Diego). The standard curve was generated by linear regression analysis of duplicate data points (Fig. 2). The SAM content in each sample then was determined from the standard curve.

Statistical methods. To determine the SAM tissue concentrations, a SAM standard curve was generated for each experiment. Duplicate data points were analyzed by linear regression analysis, yielding a linear equation of

the form

$$y = mx + b$$

where m is the slope of the standard curve, b is the y intercept, y is the amount of radioactive product formed in the enzymatic reaction utilizing the sample, and x is the quantity of SAM associated with that amount of radioactive product.

Replicates were run for each tissue or each group of tissue. The means and SEM were determined from this data. The significance between treatment groups was evaluated using two-tailed t statistics.

Results

Separation of SAM and SAH by anion exchange chromatography. Figure 1 shows the elution profile for the separation of SAM and SAH on the Dowex anion exchange resin. A single peak emerges. Examination of this peak by thin layer chromatography in 1-butanol:acetic acid:water (25:50:25) indicated only the presence of SAM. When a pure sample of SAH, well in excess of tissue concentrations (Salvatore et al., 1971), was applied to the column, no A_{254} absorbing material eluted. Thus, SAM and SAH are separated completely by anion exchange chromatography on Dowex 1-X8 as previously reported (Shapiro and Ehninger, 1966).

Figure 1 also shows that the peaks for [^3H]SAM, sample SAM as measured by absorption at 254 nm, and

sample SAM as measured by radioenzymatic assay are exactly coincident. No SAM emerges in the first 0.5 ml of column effluent. The following four 0.5-ml fractions represent the entire recoverable portion of SAM. Continued elution with distilled water results in no additional recovery of SAM.

Typical column recoveries for SAM range from 51.0 to 83.3% (Table I) and average $61.9 \pm 5.5\%$. Recoveries are not improved by additional resin preparation or further elution and seem dependent on batch variations in the resin. The column recoveries within a given experiment are highly reproducible. Variation in recoveries most often occurs between experiments, particularly when new batches of resin are employed.

Assay for SAM. Figure 2 shows a typical standard curve used in the measurement of endogenous levels of SAM. The assay is linear in the range of 0 to 2 nmol. Once the standard curve has been constructed, unknown amounts of SAM in samples can be calculated from the amount of radioactive product formed, the y intercept, and the slope of the standard curve.

The separation of SAM from SAH prior to measurement is critical since SAH is a very potent inhibitor of HIOMT (Deguchi and Barchas, 1971). Our studies indicate that the K_m for SAM is $2.04 \mu\text{M}$, while the K_i for SAH is approximately 10-fold lower, $0.22 \mu\text{M}$. Thus, it is essential that SAM be measured free of SAH contamination, since traces of SAH will lead to high estimates of the SAM content. For example, in rat adrenal gland supernatants which have not been passed through the Dowex column, the combination of SAH and SAM gives "apparent SAM" values which are 2.6 times those measured when SAH has been removed from the sample.

Measurements of tissue levels of SAM. SAM levels were determined in several rat tissues. Table II summarizes the values obtained. Spleen, liver, and kidney have the highest SAM concentrations, while heart and brain (Table III) have the lowest. In general, most of the values obtained for tissue SAM are lower than published literature values (Baldessarini and Kopin, 1966; Salvatore et al., 1971; Yu, 1978), while the dispersion about the mean

Figure 1. Separation of SAM and SAH on Dowex 1-X8, 200 to 400 mesh, 1 M NaHCO_3 . To a 1-ml sample, containing 12.1 nmol of SAM and 12.0 nmol of SAH, was added 10 μl of [^3H]SAM (specific activity, 15 mCi/mmol). A 0.5- cm^3 column of Dowex 1-X8 (200 to 400 mesh, 1 M NaHCO_3) was washed with 25 ml of freshly prepared 1 M NaHCO_3 , followed by 50 ml of distilled water. The sample was applied, followed by 10 ml of distilled water, and 0.5-ml fractions were collected. The behavior of SAM and SAH on the anion exchange resin was monitored by following the absorption at 254 nm, the radioactivity profile, and the nanomoles of SAM as measured by the HIOMT isotope dilution assay described under "Materials and Methods." Recoveries for SAM were 87%, 95%, and 86%, respectively. Thin layer chromatography of the fractions showed that SAH was absent from the SAM-containing fractions, suggesting that SAH completely binds to the column as expected.

TABLE I

S-Adenosylmethionine recovered from anion exchange columns

A 0.5- cm^3 column of Dowex 1-X8 (200 to 400 mesh, HCO_3^-) was equilibrated with 25 ml of 1 M NaHCO_3 , followed by 50 ml of distilled water. Tissue homogenates and blanks were prepared as described under "Materials and Methods." Tissue samples or samples of pure SAM were supplemented with 5 μl of [^3H]SAM tracer (specific activity, 0.4 $\mu\text{Ci}/\text{mmol}$) per 0.5 ml. One milliliter of blank was applied to the column, followed by 10 ml of distilled water. The first 0.5 ml was discarded. The second 2.0 ml was collected. Columns were re-equilibrated with NaHCO_3 and distilled water. The sample was applied and treated in the same fashion as the blank. A 0.1-ml aliquot of the 2.0-ml blank and tissue sample fraction was counted in 10 ml of Betaphase for recovery measurements.

Tissue	n	Percent Recovery
Liver	24	54.8 ± 1.7
Striata	36	52.8 ± 0.9
Adrenals	28	67.7 ± 2.3
Cerebellum	35	51.0 ± 1.0
Pineal	29	83.3 ± 2.1

Figure 2. Standard curve for the measurement of *S*-adenosylmethionine. Duplicate samples containing 0, 0.4, 0.8, 1.2, 1.6, and 2.0 nmol of unlabeled SAM were prepared and assayed for HIOMT activity as previously described. Linear regression analysis of the data points produces a curve from which tissue SAM concentrations can be calculated using the *y* intercept and the slope of the line.

is less than or equivalent to that encountered by other assay methods.

Measurement of SAM in rat brain. Analysis of SAM levels in several brain regions was conducted (Table III). Septum, cerebral cortex, and striatum had the highest SAM values, although these regions would be considered low in comparison to the values observed in peripheral tissues. Thalamus, pons-medulla, and hypothalamus were the brain regions with the lowest SAM content.

Effects of hypophysectomy on SAM levels. Our initial interest in studying the control of SAM levels was prompted by the possible role of this compound as a regulator of *in vivo* methyltransferase levels. We have shown previously that hypophysectomy decreases PNMT activity in rats to half the values observed in control animals and nearly abolishes pineal HIOMT activity. Although administration of ACTH or glucocorticoids does not elevate enzyme activity above normal values in control animals, these compounds do restore the activity of both enzymes toward control levels in hypophysectomized animals (Ciaranello, 1978; Sandrock et al., 1980). SAM administration to hypophysectomized rats also restores the levels of the two enzymes and

stabilizes both against enzymatic degradation as well. These findings led us to propose a model in which SAM binds directly to the methyltransferases and stabilizes them against intracellular degradation (Ciaranello, 1978; Masover et al., 1979). A necessary condition of this model is that SAM must be under glucocorticoid control at least in the pineal and adrenal. In the adrenal, SAM levels fall dramatically after hypophysectomy and are partially restored by dexamethasone or ACTH administration

TABLE II

Concentration of S-adenosylmethionine in rat tissue

A 0.5-cm³ column of Dowex 1-X8 (200 to 400 mesh, HCO₃⁻) was equilibrated with 1 M NaHCO₃, followed by distilled water. Male Sprague-Dawley rats (160 to 200 gm) were killed by cervical dislocation and the various organs were removed. Tissue samples were homogenized in 5 vol of 10% trichloroacetic acid, 0.05 M hydrochloric acid and centrifuged at 3000 rpm for 30 min at 4°C. Blanks of TCA-HCl and tissue samples were washed three times with twice their volume of ether saturated with 0.05 M HCl. Residual ether was evaporated under N₂ gas at 0°C and stored overnight at 4°C. A 0.200-ml aliquot of sample and blank was diluted to 1.0 ml with distilled water. To the sample was added 5 μl of tracer [³H]SAM (specific activity, 0.4 μCi/mmol) per 0.5 ml of original extract. Blanks were applied to the columns, followed by 10 ml of distilled water. The first 0.5 ml of effluent was discarded and the second 2.5 ml was collected. The column was re-equilibrated with NaHCO₃ and distilled water. Samples were run similarly to blanks. A 1.000-ml aliquot was assayed for HIOMT activity. SAM content was calculated from the *y* intercept and the slope of the SAM standard curve. Samples were run in triplicate and standard curves generated with each experiment.

Tissue	μg/gm
Spleen	30.1 ± 3.0
Liver	24.9 ± 2.3
Kidney	21.3 ± 1.7
Adrenals	16.1 ± 1.7
Lung	14.7 ± 1.6
Heart	7.7 ± 2.6

TABLE III

Regional brain distribution of S-adenosylmethionine in rats

Twelve animals were used per group. Animals were sacrificed by cervical dislocation and pineals and brains were removed. The brain was dissected regionally on ice, and tissue samples were frozen until assay. Each part was weighed prior to homogenization in 4 vol of distilled water. To each aqueous homogenate was added 1 vol of 50% TCA, 0.25 M HCl. Following mixing, samples were centrifuged and the supernatants were washed three times with twice their volume of ether saturated with 0.05 M HCl. Residual ether was evaporated with a gentle stream of nitrogen gas, and samples were stored at 0 to 4°C overnight. SAM was measured as described under "Materials and Methods."

Brain Region	Weight mg	μg/gm
Pineal	— ^a	0.12 ± 0.04
Striatum	125.4 ± 3.0	7.32 ± 0.83
Hypothalamus	28.0 ± 1.6	1.67 ± 0.37
Cortex	731.6 ± 11.4	7.39 ± 0.25
Cerebellum	273.8 ± 3.8	2.89 ± 0.36
Thalamus	174.7 ± 6.5	1.95 ± 0.33
Hippocampus	125.6 ± 2.6	6.01 ± 0.40
Midbrain	112.1 ± 6.5	4.32 ± 0.40
Septa	15.4 ± 0.7	12.56 ± 0.89
Pons-medulla	189.4 ± 7.2	1.86 ± 0.38

^a Values for pineal are expressed in micrograms per gland.

TABLE IV

S-Adenosylmethionine levels in adrenals from control, hypophysectomized, and dexamethasone-treated rats

Twelve animals were used per group. Animals were injected with 4 mg/kg of dexamethasone phosphate (i.p.) daily for 1 week. Adrenals were removed and homogenized in 200 μ l of distilled water. A 5- μ l aliquot was assayed for PNMT activity. To the remaining homogenate was added 50 μ l of 50% TCA, 0.25 M HCl. The sample was centrifuged for 1.5 min and the resulting supernatant was washed three times with twice its volume of ether saturated with 0.05 M HCl. Residual ether was removed with N₂ gas and the sample was stored overnight at 0 to 4°C. SAM content was measured as described under "Materials and Methods."

	PNMT Activity	SAM
	units/pair	μ g/pair
Control	1.60 $\pm$ 0.01	0.49 $\pm$ 0.08
Hypophysectomized	0.45 $\pm$ 0.01 ^a	0.23 $\pm$ 0.05 ^b
Hypophysectomized + dexamethasone	0.87 $\pm$ 0.01 ^c	0.60 $\pm$ 0.09 ^d

^a $p < 10^{-9}$, significant compared to control.

^b $p < 0.02$, significant compared to control.

^c $p < 10^{-9}$, significant compared to hypophysectomized rats.

^d $p = 0.12$, not significantly different from control; $p < 10^{-3}$, significant compared to hypophysectomized rats.

TABLE V

HIOMT activity and SAM concentration in pineal of control, hypophysectomized, and dexamethasone-treated hypophysectomized rats

Dexamethasone-treated animals were administered the drug at a daily dosage of 4 mg/kg (i.p.) for 1 week. Animals were sacrificed and the pineals were removed. Each gland was homogenized in 250 μ l of distilled water. A 50- μ l portion was assayed for HIOMT activity and SAM content was measured as previously described.

	HIOMT Activity	SAM
	units/gland	μ g/gland
Control	85.3 $\pm$ 10.4	0.35 $\pm$ 0.03
Hypophysectomized	31.1 $\pm$ 4.7 ^a	0.15 $\pm$ 0.01 ^b
Hypophysectomized + dexamethasone	51.4 $\pm$ 6.7 ^c	0.19 $\pm$ 0.01 ^d

^a Significant with respect to control at $p < 0.001$.

^b Significant with respect to control at $p < 0.0001$.

^c Significant with respect to hypophysectomized rats at $p = 0.03$.

^d Significant with respect to hypophysectomized rats at $p = 0.03$.

(Table IV). Both the decline and restoration of SAM levels correspond with the changes in PNMT activity. Similar results have been obtained in the pineal (Table V). These results suggest that adrenal cortical hormones play an important role in the maintenance of SAM levels in both the adrenal and pineal.

The modulation of SAM levels by glucocorticoids in the pineal and adrenal indicates that these hormones might be an essential component in other SAM-dependent methylations. In most cases (e.g., brain phospholipid methylation), a role for glucocorticoids has not been demonstrated. The importance of glucocorticoids in maintaining SAM levels in pineal and adrenal, however, suggested that the role of glucocorticoids should be investigated in other SAM-containing tissues. Consequently, we studied SAM levels in the liver and in several brain regions.

The brain regions chosen were those which actively

incorporate and bind glucocorticoids. Various regions of the brain are known to contain glucocorticoid receptors. However, the regional distribution of glucocorticoid receptors varies greatly, depending on the specific glucocorticoid radioligand used in the receptor determinations (McEwen, 1979). When [³H]corticosterone is used as the receptor-labeling ligand, the highest corticoid binding in the rat is observed in the hippocampus, septum, and amygdala, with neurons being the primary cells labeled. In contrast, [³H]dexamethasone predominantly labels glucocorticoid receptors in the hypothalamus, amygdala, septum, hippocampus, and cortex.

Figure 3 shows the results of these studies in which SAM levels were determined in brain regions of hypophysectomized rats, some of which were treated with dexamethasone. The results are quite complicated and suggest that glucocorticoids are not the sole regulators of SAM levels. Three patterns emerge from the data. In the striatum and midbrain, hypophysectomy causes a decrease in the SAM content of these tissues, while dexa-

Figure 3. Regional distribution of S-adenosylmethionine in rat brain from control, hypophysectomized, and dexamethasone-treated animals. Each experimental group consisted of 12 animals. Dexamethasone was administered daily at a dose of 4 mg/kg (i.p.) for 1 week. Animals were sacrificed by cervical dislocation. Brains were removed and regionally dissected. The tissue was stored frozen at -70°C until use and analyzed for SAM as previously described. *, A significant difference from control; **, a significant difference from hypophysectomized animals.

methasone administration restores the nucleoside toward control values. This is a response similar to that seen in adrenal and pineal. In contrast, hypophysectomy increases SAM levels in the thalamus, hypothalamus, hippocampus, and cerebellum, but dexamethasone administration does not alter SAM levels further. The SAM content of the cortex, septum, pons-medulla, and liver is unaffected either by hypophysectomy or dexamethasone administration.

Discussion

SAM quantitation by the procedure that we have described in this paper provides rapid and accurate measurement of this nucleoside. The values that we obtained for peripheral tissue and regional brain SAM concentrations tend to be lower than those obtained by existing methods with errors less than or comparable to the most sensitive method. These lower SAM concentrations are consistent with the complete separation of SAM from SAH. SAH is a competitive inhibitor of the methyltransferases used in the radioisotope dilution assays for SAM measurement. Hence, its presence would lead to high estimates of SAM. The SAH bound to the anion exchange resin can be eluted from the column with 0.1 N NaCl (Shapiro and Ehninger, 1966) and measured separately using a modification of the same radioenzymatic assay that we have described (D. L. Wong, E. L. Zager, and R. D. Ciaranello, unpublished results).

Existing SAM assays have dealt with the problem of SAH contamination in samples in a variety of other ways: dual label radioenzymatic assay combined with mathematical analysis (Baldessarini and Kopin, 1966), single label radioenzymatic assay using radiolabeled SAM as an internal standard combined with mathematical analysis (Yu, 1978), or separation by thin layer or paper chromatography and measurement by absorption at 254 nm (Salvatore et al., 1971). The latter methodology, although appropriate to the separation procedure, is less sensitive than the enzymatic assays for SAM measurement. Even in the case of the two enzymatic assays, there was concern that the limits of the assays might obviate the detection of small tissue fluctuations in SAM. Although these protocols may account for SAH contamination, there is reason to believe that *in vivo* regulation may occur as a consequence of the relative proportions of SAM and SAH. Hence, a procedure was required which would separate SAM and SAH so that their concentrations could be determined independently. A method which separates and measures both SAM and SAH by reverse phase C18 high pressure liquid chromatography using gradient elution has appeared recently (Hoffman et al., 1979). However, this methodology requires the use of expensive equipment, is time consuming (80 min/run), and most importantly, requires gram quantities of tissue. Our procedure offers the advantage of being quick, simple, sensitive, and inexpensive.

This new procedure for SAM assay was used to test the possibility that SAM may regulate the intracellular proteolysis of PNMT and HIOMT. We have shown that both adrenal and pineal SAM levels decrease after hypophysectomy and are restored by dexamethasone administration. These results provide further evidence that

glucocorticoids mediate the *in vivo* regulation of SAM in the adrenal and pineal and that SAM, in turn, regulates the *in vivo* degradation of both PNMT and HIOMT.

Perhaps even more interesting are our findings which suggest that hormonal control of SAM varies markedly in various regions of the brain and in peripheral tissues. In some peripheral tissues, and in some areas of the central nervous system, adrenal, pineal, striatum, and midbrain, SAM seems to be under glucocorticoid influence in that SAM levels decrease after hypophysectomy and are restored by dexamethasone administration. In other regions and tissues, thalamus, hypothalamus, hippocampus, and cerebellum, only a partial effect is observed or no effect at all. Although glucocorticoids may constitute one regulatory control of SAM, our results suggest that other controls exist as well. Some of these controls also may be endocrine factors. For example, in tissues where glucocorticoids are ineffective in reversing SAM changes in hypophysectomized animals, cortex, septum, pons-medulla, and liver, other pituitary factors may be important besides ACTH, the pituitary hormone responsible for glucocorticoid induction. Any of the pituitary hormones are possible candidates as these hormones are distributed both centrally and peripherally in other regions besides their classical target organ regions and are known to be involved in other non-target organ-related functions. In tissues where hypophysectomy and glucocorticoids are ineffective in altering SAM concentrations, the pituitary and its hormones probably are not involved in SAM regulation. However, control could occur centrally via the hypothalamic releasing hormones which have other biological activities in addition to the induction of the pituitary hormones.

Nonendocrine factors, particularly neuronal influences, also may regulate *in vivo* levels of SAM. Alternatively, a combination of hormonal and neuronal factors may be important.

This work emphasizes the multiplicity of factors involved in SAM regulation. Future work should concentrate on the further identification of the factors involved in SAM regulation. This should include an examination of other glucocorticoids, such as corticosterone, pituitary or hypothalamic hormones, and neural factors. In addition, it will be important to identify the biochemical sites

TABLE VI

Effect of hypophysectomy and dexamethasone on PNMT and MAT

Twelve animals were used per group. Hypophysectomized rats were administered dexamethasone phosphate daily for 1 week at a dose of 4 mg/kg (i.p.). Animals were sacrificed, and the adrenals were removed. The tissue was homogenized in 0.03 M potassium phosphate, pH 6.0, 2.5 mM DTT. PNMT and MAT activities were measured simultaneously. PNMT was assayed as previously described. MAT was assayed by a modification of the procedure of Chou and Lombardini (1972).

	PNMT Activity	MAT Activity
	units/pair	
Control	0.76 ± 0.04	0.0270 ± 0.0020
Hypophysectomized	0.03 ± 0.01 ^a	0.0025 ± 0.0003 ^a
Hypophysectomized + dexamethasone	0.92 ± 0.22 ^b	0.0075 ± 0.0013 ^b

^a Significantly different from control at $p \leq 3 \times 10^{-8}$.

^b Significantly different from hypophysectomized rats at $p \leq 10^{-3}$.

of action on which these control systems are operant. Current studies are focusing on hormonal and nonhormonal regulation of methionine adenosyltransferase (MAT) and *S*-adenosylhomocysteine hydrolase, two enzymes involved in SAM metabolism. Preliminary evidence suggests that glucocorticoids regulate the levels of adrenal methionine adenosyltransferase, the enzyme responsible for SAM biosynthesis (Table VI).

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