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SHORT COMMUNICATIONS

Kinetics of the glucocorticoid-mediated induction of phenylethanolamine *N*-methyl transferase in the hypophysectomized rat

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PHENYLETHANOLAMINE-*N*-METHYL transferase (PNMT) is an enzyme localized to the adrenal medulla[1] and brain,[2,3] which catalyzes the conversion of noradrenaline to adrenaline. Considerable evidence indicates that adrenal PNMT is regulated by the pituitary–adrenal axis.[4] Hypophysectomy results in a marked decrease in PNMT activity, and treatment with ACTH or glucocorticoids restores activity to normal levels.[4] This steroid-mediated increase in enzyme activity is prevented by the protein-synthesis inhibitor, puromycin,[4] suggesting that ongoing protein synthesis is necessary for the rise in PNMT activity. This induction may, in turn, be due to an increase in the rate of synthesis of PNMT, or to a decrease in the rate of degradation, or to both.

This communication reports kinetic experiments which suggest that dexamethasone induces PNMT in the hypophysectomized rat by increasing the rate of enzyme synthesis. Additional observations, demonstrating increased PNMT in normal rats after reserpine treatment suggests a second, nonsteroid mechanism involved in PNMT regulation.

Female, Sprague–Dawley, 180–200 g rats (Hormone Assay Laboratories, Chicago, Ill.) were offered Purina lab chow and water *ad lib.*, and the diet of hypophysectomized rats was supplemented with 1% saline and oranges. Experiments on hypophysectomized rats were begun on day 17–21 after operation with injection of the synthetic glucocorticoid, dexamethasone (Decadron; Merck, Sharp & Dohme).

Rats were killed by cervical dislocation and adrenal PNMT was assayed by a modification of the method of Axelrod,[1] using phenylethanolamine as substrate and ^{14}C-S-adenosylmethionine (specific activity, 55 mc/m-mole; New England Nuclear) as methyl donor.

In the studies of PNMT turnover, the data were plotted semilogarithmically according to the method of least squares weighted for the number of rats and standard error. The slope of the decline in PNMT after cessation of steroid treatment was used to calculate the half-life according to the equation: $T_{-1/2} = 0{\cdot}693/K$.

To determine the optimal dose of dexamethasone for PNMT induction in the hypophysectomized rat, varying amounts were administered for 3 days. Peak enzyme activity was obtained with 0·5 mg/kg (Table 1). Higher doses resulted in debility and a submaximal enzyme response.

To determine whether this induction is dependent on increased enzyme synthesis or decreased degradation, a kinetic analysis of the induction was performed. Schimke *et al.*[5] have demonstrated that an increase in the rate of enzyme synthesis results in an exponential rise in enzyme activity to a new steady state plateau. A decrease in the rate of enzyme degradation approaching zero as a limit results in a linear rise in enzyme activity to a theoretically infinite level.[5] To distinguish between these alternatives, repeated daily optimal doses of dexamethasone were administered. PNMT activity increased to an elevated plateau level (Fig. 1), which is normal for the intact rat. These results are consistent with the hypothesis that increased enzyme synthesis, and not decreased degradation, was responsible for the rise.

The "apparent"* half-life of PNMT was measured by following the decay of enzyme activity from plateau levels induced by dexamethasone. Hypophysectomized rats were treated with daily doses of dexamethasone for 11 days, at which time steroid administration was stopped and the rate of decline of PNMT activity was determined. Enzyme activity decreased with a half-life of 6 days, indicating a relatively slow turnover of PNMT.

These observations indicate that a prolonged period of time, measured in days, is required for the steroid induction of PNMT in the hypophysectomized animal, seemingly excluding this mechanism as critical in the acute stress response. These data further imply that continued steroid administration

* The term "apparent" is used, since the rate of decline of PNMT activity was determined without specifically blocking protein synthesis. The hypophysectomized rats were unable to tolerate prolonged treatment with inhibitors of protein synthesis. Thus, the apparent calculated half-life will tend to be greater to the extent that residual protein synthesis occurs.

TABLE 1. EFFECT OF VARYING DOSAGES OF DEXAMETHASONE ON PNMT ACTIVITY*

Dexamethasone (mg/kg)	PNMT activity (units/adrenal ± S.E.M.)
0 (Saline)	2·73 ± 0·18
0·005	2·92 ± 0·21
0·05	4·53 ± 0·25
0·50	5·79 ± 0·14
5·0	5·20 ± 0·11

*Hypophysectomized rats were given dexamethasone intraperitoneally in the doses shown and their PNMT levels determined as described in Methods. Each value represents the mean and standard error of six to eight animals. One unit of enzyme activity represents the formation of 1 mμmole *N*-methyl-^{14}C-phenylethanolamine/hr.

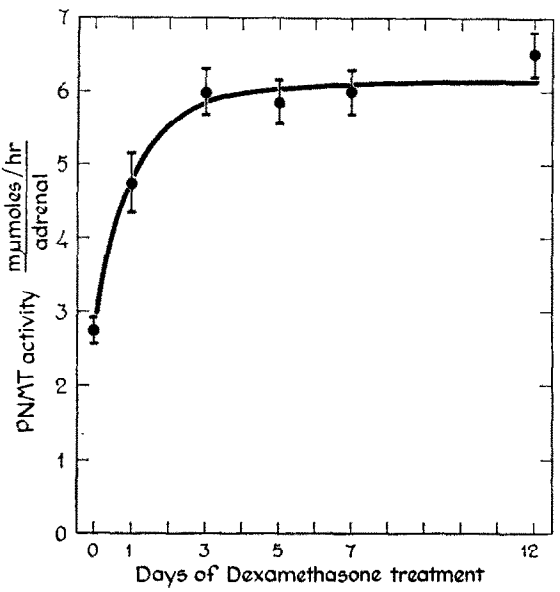

FIG. 1. Response of rat adrenal PNMT to dexamethazone. Hypophysectomized rats were treated with 0·5 mg/kg of dexamethasone daily and their PNMT levels determined on the days shown. Groups consisted of from 6 to 8 animals per point; data are expressed as mean ± standard error.

results only in a limited induction of PNMT to normal plateau levels. Previous work[6,7] had demonstrated that with continuous intense activation of the adrenal pituitary axis much longer periods of time are required for PNMT to rise above control values in the normal rat.

It became of interest to determine if a nonsteroid mechanism altering PNMT activity was operative in the intact rat. Since noradrenaline is known to inhibit PNMT activity and since reserpine has been shown to increase adrenal tyrosine hydroxylase[8] and dopamine-β-hydroxylase,[9] other enzymes involved in adrenaline synthesis, the effect of reserpine on PNMT activity was determined. Normal rats were treated with reserpine and killed at varying times thereafter. Reserpine treatment resulted in a significant elevation of PNMT activity within 7 days in the intact rat (Fig. 2). This is consistent with findings which have been recently reported.[9]

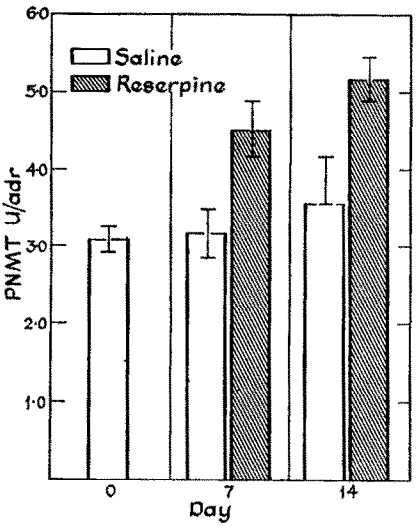

FIG. 2. Response of intact rats to reserpine treatment. Animals used were Sprague–Dawley females, 180–200 g, obtained from Simonsen Laboratories, Gilroy, Calif. These animals have normal PNMT levels, approximately 60 per cent of normal rats from Hormone Assay Laboratories. We are unable to distinguish any other differences in the PNMT response of the two animal groups. The reserpine regimen was as follows: in the first injection, rats received 5 mg/kg of reserpine; thereafter they received 2 mg/kg on alternate days for 14 days. PNMT activity is expressed as units (millimicromoles of product formed per hour) per whole adrenal. Each bar represents the mean ± standard error of from six to eight animals.

These observations are consistent with a model in which at least two different mechanisms regulate adrenal PNMT activity. Adrenal corticoids appear to maintain PNMT at normal plateau levels by maintaining the rate of enzyme synthesis. Continued steroid treatment, however, does not elevate PNMT above normal control activity, nor does dexamethasone administration to intact rats increase PNMT activity[4] (Table 2). Reserpine administration, on the other hand, results in elevation

TABLE 2. EFFECTS OF CHRONIC DEXAMETHASONE TREATMENT IN THE INTACT RAT*

Treatment	Adrenal wt. (mg/pair)	PNMT (U/adrenal)
5-Day dexamethasone	38·3 ± 1·4†	5·29 ± 0·33
5-Day saline	59·5 ± 2·9	5·06 ± 0·55

* Groups of rats were treated with dexamethasone, 0·5 mg/kg daily, i.p., or an equivalent volume of saline, for 5 days. Each group contained six animals; values shown are the means and standard errors of the results.
† $P < 0·01$ differs from control.

of PNMT above the steroid-maintained plateau levels. These findings are consistent with those of Thoenen *et al.*,[10] who described increased PNMT activity after the administration of 6-hydroxydopamine, a compound which destroys sympathetic nerve endings. This rise in PNMT activity was prevented by denervation and was attributed to an increased frequency of neural impulses. Similarly, Kvetnansky *et al.*[11] have demonstrated increased adrenal PNMT activity after immobilization stress, which is dependent on adrenal innervation. Our data with reserpine lends further support to a model in which increased nerve firing is followed by elevation of PNMT activity.

It is apparent, however, that both the steroid and neural mechanisms described represent long-term regulation, and cannot play a role in short-term adrenal medullary responses to acute stress. The half-life of PNMT in the hypophysectomized rat is quite long, and is probably much longer in the intact rat.[6,7] These findings suggest that the elevation of PNMT in the intact animal, whether mediated by hormonal or neural factors, probably finds its biologic role in chronic stress situations.

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Department of Psychiatry,
Stanford University School of Medicine,
Stanford Calif. 94305 *and*
Laboratory of Clinical Science,
National Institute of Mental Health,
Bethesda, Md. 20215, *U.S.A.*

ROLAND D. CIARANELLO
IRA B. BLACK

REFERENCES

1. J. AXELROD, *J. biol. Chem.* **237**, 1657 (1962).
2. R. D. CIARANELLO, R. BARCHAS, G. BYERS, D. W. STEMMLE and J. D. BARCHAS, *Nature, Lond.* **221**, 368 (1969).
3. L. POHORECKY, M. ZIGMOND, H. KARTEN and R. J. WURTMAN, *J. Pharmac. exp. Ther.* **165**, 190 (1969).
4. R. J. WURTMAN and J. AXELROD, *J. biol. Chem.* **241**, 2301 (1966).
5. R. T. SCHIMKE, E. W. SWEENEY and C. M. BERLINER, *Biochem. biophys. Res. Commun.* **15**, 214 (1964).
6. J. VERNIKOS-DANELLIS, R. CIARANELLO and J. BARCHAS, *Endocrinology* **83**, 1357 (1968).
7. R. D. CIARANELLO, J. D. BARCHAS and J. VERNIKOS-DANELLIS, *Life Sci.* **8**, 401 (1969).
8. R. A. MUELLER, H. THOENEN and J. AXELROD, *J. Pharmac. exp. Ther.* **169**, 74 (1969).
9. P. MOLINOFF, S. BRIMIJOIN, R. WEINSHILBOUM and J. AXELROD, *Proc. natn. Acad. Sci. U.S.A.* **66**, 453 (1970).
10. H. THOENEN, R. A. MUELLER and J. AXELROD, *Biochem. Pharmac.* **19**, 669 (1970).
11. R. KVETNANSKY, V. K. WEISE and I. J. KOPIN, *Endocrinology* **87**, 744 (1970).

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"Activation" of nitrofurazone in animal tissues

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DERIVATIVES of 5-nitrofuran have been used clinically as antibacterial agents for a number of decades.[1,2] Studies on the mode of action of these compounds have shown that reductive metabolism greatly increases their toxicity to bacteria. Mutants with decreased levels of nitrofurazone-reductase are resistant to the drug[3,4] suggesting that metabolic "activation" is required. Early toxicological studies showed that severe liver and kidney damage was produced in rodents by injection of high but sub-lethal doses of nitrofurazone.[5] Spermatogenic arrest follows administration of several nitrofurans[6,7] and Friedgood and Green[8] reported marked atrophy of testes following oral doses of nitrofurazone. The same workers also observed weak antitumor activity against fibrosarcoma in mice,[8] an observation which led to the experimental treatment of primary and metastatic seminoma with nitrofurazone.[9,10]

More recently it has been discovered that other nitrofuran derivatives are powerful carcinogens.[11–13] Results published within the last year show that nitrofurazone can induce transplantable mammary tumors.[14] Nitrofurantoin does not appear to be carcinogenic.

Like certain other carcinogenic nitro compounds,[15,16] nitrofuran derivatives are known to undergo reductive metabolism in animal tissues.[17] We have recently shown that intermediates formed during the reduction of nitrofurazone react with serum albumin to form derivatives which are stable to dilute acid and to prolonged dialysis against 8 M urea[4] and which have altered electrophoretic mobility.*

* D. R. McCalla, A. Reuvers and C. Kaiser, unpublished results.