In addition, certain synthetic homopolyribonucleotides are capable of inhibiting DNA synthesis in mammalian tissues and tumors (*11*). It would seem important to determine whether this inhibition is accomplished by competition of the exogenous homopolyribonucleotide with the templates of the cell in a similar manner as is observed here with DNA polymerase and purified calf thymus DNA.

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Hydrocortisone-Mediated Increase of Norepinephrine Uptake by Brain Slices

Abstract. *Slices of cerebral cortex from the adult rat were incubated with and without hydrocortisone succinate, and their subsequent uptake of isotopically labeled norepinephrine was measured. Preincubation with hydrocortisone resulted in a statistically significant increase in the amount of exogenous norepinephrine taken up by the slices. Preincubation with corresponding concentrations of sodium succinate was without effect. The principal effect of the hydrocortisone-mediated increase in norepinephrine concentration is apparently on an active transport mechanism.*

Catecholamine and steroid hormones have both been implicated as regulators of mood and behavior in humans and experimental animals, and, although their mode of interaction is unclear, there is much work that suggests that cortisol has an important "permissive" role in mediating the physiological actions of the catecholamines (*1–3*). A significant number of patients with severe depressions, in which a central nervous system deficiency of functional norepinephrine (NE) may exist, have elevated concentrations of plasma hydrocortisone, excrete greater than normal amounts of cortisol metabolites, and exhibit dexamethasone resistance (*4*). Since tissues such as heart and brain are able to accumulate exogenous NE against a gradient by an energy-dependent transport mechanism (*5, 6*), such preparations offer the possibility for examining the presently ill-defined relation between hydrocortisone and the disposition of the catecholamines. We present here data indicating that, in the presence of cortisol, the uptake of exogenous NE by slices of cerebral cortex is significantly increased and that the principal effect of the steroid hormone is on an active transport process or "pump" mechanism.

Adult rat cerebral cortex "first" slices (50 to 60 mg) were prepared as described by McIlwain (*7*) and then incubated at 37°C in 2.5 ml of a 10 m*M* solution of glucose and Krebs-Ringer bicarbonate (Fisher Scientific) in an oxygen atmosphere for 30 minutes with and without the indicated amounts of hydrocortisone succinate (Upjohn) or sodium succinate (Calbiochem). Various concentrations of *dl*-[7-^{3}H]norepinephrine (10 c/mmole; New England Nuclear) were then added, and the incubation was continued for another 15 minutes at which time the reaction was terminated by placing the mixture in an ice bath. The incubation medium was removed by suction. The slices were washed twice with 2.5 ml of cold medium and homogenized in 0.2*N* HCl. The radioactivity of a portion of the homogenate was measured, and another portion was adsorbed onto Al_2O_3 (M. Woelm Co.) by a modification of the method of Anton and Sayre (*8*). The catechols containing no amines were extracted from the acid eluate with ethyl acetate (*8–10*). A portion of the aqueous phase was dissolved in 3.0 ml of ethanol and was added to 10 ml of scintillator solution containing 2,5-diphenyloxazole (5 g/liter) and 1,4-*bis*-2-(4-methyl-5-phenyloxazoyl)-benzene (2.5 g/liter). The radioactivity was measured in a Packard liquid scintillation spectrometer. Control slices were included in each experiment, and corrections were made for recovery losses on alumina adsorption. When the effect of the inhibitor ouabain was tested, the incubations with hydrocortisone and the appropriate controls were conducted at 37°C for 30 minutes, at which time the [^{3}H]NE was added, and the incubation mixture was made 1×10^{-4}*M* with respect to ouabain. As before, the subsequent incubation was carried out for 15 minutes at 37°C. For the determination of uptake of NE at 0° to 4°C the initial incubations, with and

Table 1. Uptake of NE by slices of rat cerebral cortex. The NE concentration was 29 ng/ml in a 10 m*M* solution of dextrose and Krebs-Ringer bicarbonate incubation medium. Samples were preincubated with the indicated additives for 30 minutes at 37°C, [^{3}H]NE was added, and the incubation was carried out for another 15 minutes. The incubation mixture was then chilled in ice to stop the reaction. In some experiments, as indicated, the 15-minute incubation period was done at 0° to 4°C (the preincubation in all cases was done at 37°C). Results are averages of 3 to 10 experiments.

Incubation medium additive			Temp (°C)	[^{3}H]NE incorporation		In/Ex
Sodium succinate (*M*)	Hydrocortisone succinate (*M*)	Ouabain (*M*)		Amount per 15 minutes (ng/g)	[^{3}H]NE / [^{3}H]Total	
			37	67.15 ± 8.01	0.79 ± 0.05	2.32
5×10^{-5}			37	66.00 ± 6.44	0.84 ± 0.09	2.28
	4.2×10^{-5}		37	86.42 ± 12.30	0.86 ± 0.05	2.98
	8.4×10^{-5}		37	102.60 ± 12.36	0.85 ± 0.03	3.54
1.0×10^{-4}		1×10^{-4}	37	37.23 ± 9.39	0.68 ± 0.08	1.28
	8.4×10^{-5}	1×10^{-4}	37	27.05 ± 5.86	0.78 ± 0.13	0.93
1.0×10^{-4}			0–4	16.32 ± 3.28	1.16 ± 0.05	0.56
	8.4×10^{-5}		0–4	17.70 ± 3.78	1.03 ± 0.23	0.61

without hydrocortisone, were conducted at 37°C for 30 minutes. The reaction mixtures were then placed in an ice bath, [^{3}H]NE was added, and after 15 minutes the procedure described above was followed.

At a constant concentration of NE (29 ng per milliliter of incubation medium), the average uptake of NE by rat cerebral cortex slices incubated in medium alone during the 15-minute period was 67.15 ng per gram of tissue (Table 1). Under similar conditions Dengler *et al.* (*5*) reported an average uptake of 46.0 ng per gram of slice of cat cerebral cortex. When the incubation medium was made 4.2×10^{-5} and $8.4 \times 10^{-5}M$ with respect to hydrocortisone succinate, the uptake of NE rose to 86.4 and 102.6 ng per gram of slice, respectively—a statistically significant increase ($P < .005$) (Table 1). The ratio of the concentration of NE in the slice (In) to that in incubation medium (Ex) was 2.32 for the medium alone, while for the two concentrations of hydrocortisone used, it was 2.98 and 3.52, respectively (Table 1). Varying the sodium succinate concentrations between 5×10^{-5} and 1.5×10^{-4} did not affect the NE uptake. When $1 \times 10^{-4}M$ ouabain was added to the incubation mixture, the ratio of In to Ex of both the control and the hydrocortisone samples was approximately 1.0, or that of passive diffusion (Table 1). These results suggest that the hydrocortisone-mediated increase of NE concentration in the cerebral cortex slices is linked to a functionally active transport system (*5*). Similarily, when the NE uptake was measured at 0° to 4°C, the NE concentrating mechanism was inoperative, and NE uptake was not stimulated by prior incubation with hydrocortisone (Table 1).

These experiments show that the effect of hydrocortisone is not an artifact of tissue swelling induced by prior incubation with the steroid hormone. Comparison of the ratios of [^{3}H]NE to total ^{3}H in the slices incubated with and without hydrocortisone (Table 1) shows that any effect of hydrocortisone on a degradative process is either absent or too small to account for the magnitude of the increase of NE in the slices due to hydrocortisone. To insure that the hydrocortisone effect was not due to an increased adherence of [^{3}H]NE to the slices, the slices were washed as described above. To check the effectiveness of the washing, the ratio of the amount of total ^{3}H in the second wash (2.5 ml) to that in the 50- to 60-mg slice was calculated and was found to be 0.051 ± 0.006 ($N = 8$); that is, on a concentration basis the ratio of the amount of NE in the tissue to that in the second wash was approximately 1000 : 1, an indication that the two washes were adequate to remove NE that was not firmly bound.

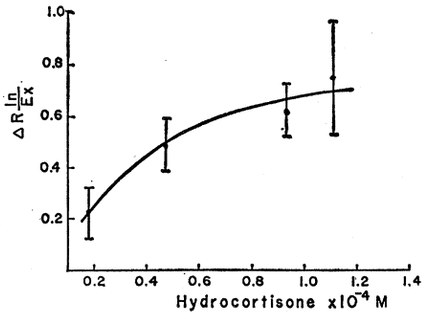

Fig. 1. The relation between the concentration of hydrocortisone and the uptake of NE by rat cerebral slices from the incubation medium. The difference (△ In/Ex) is the ratio of the concentration of NE in the cerebral slices (In) to that in the media (Ex) of the controls subtracted from that of the samples incubated with hydrocortisone.

The relation between the hydrocortisone concentration in the incubating medium and the uptake of NE by the slices is shown in Fig. 1, which is based on experiments where the NE concentration varied from 20 to approximately 60 ng/ml; in this concentration range there is an approximately linear relation between slice uptake and exogenous concentration of NE for a 15-minute period. The hydrocortisone concentration (Fig. 1) varied from 1.62×10^{-5} to $1.62 \times 10^{-4}M$, and was plotted against the difference (Δ) between the ratio, In/Ex, for the controls and that for the slices incubated with hydrocortisone. This method of comparison was chosen because of the different concentrations of NE in the medium and because of some variation in the absolute amounts of NE taken up by different slice preparations. Touchstone *et al.* (*10*) found that the concentration of cortisol in brain (4 μg/g; $7.96 \times 10^{-6}M$) was considerably higher than that in plasma, (0.15 μg/ml) or in cerebrospinal fluid. This endogenous concentration of hydrocortisone is within an order of magnitude of the amount of steroid which effected an increase in NE uptake in our experiments. Also, cortisol crosses the blood-brain barrier, is selectively retained by certain structures, and may disappear more slowly from brain than from other tissues (*11*).

The uptake of NE is one of the principal ways by which the released amine is inactivated (*12*), NE uptake stimulates the production of cyclic adenosine monophosphate (*13*), and release and reuptake may relate to the regulation of catecholamine synthesis. For these reasons the increased uptake of NE that occurs in the presence of hydrocortisone may have important functional significance. In reviewing the relation between the biogenic amines and adrenal corticoids, Ramey and Goldstein (*1*) concluded from indirect evidence that norepinephrine and epinephrine function as a physiological unit with cortisol. This conclusion is supported by our findings.

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