*A SUSTAINED EFFECT OF ELECTROCONVULSIVE SHOCK ON THE TURNOVER OF NOREPINEPHRINE IN THE CENTRAL NERVOUS SYSTEM OF THE RAT**

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Although the etiology of the depressive illnesses is unknown, evidence acquired over the past decade has suggested to many authors the hypothesis that these states may result, in part, from a diminished functional activity of norepinephrine in certain regions of the brain. Neurochemical, pharmacological, and behavioral studies have provided a number of findings compatible with this hypothesis.[1] Reserpine, a hypotensive and tranquilizing agent which characteristically depletes the brain of norepinephrine and other amines,[2] has been found in a significant proportion of patients receiving it to result in a state closely resembling endogenous depression.[3] On the other hand, a number of drugs which elevate mood and have been found of value in the treatment of depression appear to act on central norepinephrine in ways which could increase its physiologically active concentration,[4] either by inhibiting the enzyme responsible for its presynaptic deamination, by favoring its release, or by inhibiting its re-uptake, presumably at central synapses.

Introduced by Cerletti and Bini in 1938,[5] two decades before modern drug treatments, electroconvulsive therapy has proved to be the most effective form of treatment of depressive illness: extensive clinical experience[6] and a number of controlled studies[7] agree that approximately 80 per cent of depressed patients experience significant, sustained improvement or disappearance of symptoms in the course of a series of electroconvulsive shocks given at intervals of one to a few days. Although there is general agreement on its efficacy, there is little knowledge with which to formulate an explanation of its mechanism of action. Central and peripheral biochemical effects have been observed which are inconstant or attributable to the acute functional disturbances attending the shock itself.[8]

If a deficiency of norepinephrine were involved in depressive illness, one would expect some change induced by electroconvulsive shock in the availability of this amine in the brain, compatible with one or another of the effects reported for the antidepressant drugs. Rosenblatt and co-workers[9] have reported evidence for an increased permeability of the blood:brain barrier to norepinephrine after electroshock, but the elevation in blood norepinephrine which follows such treatment[10] is neither sufficiently great nor prolonged to affect brain concentrations appreciably. Schatalova and Antonov[11] have reported no change in brain norepinephrine concentration after a single electrically induced convulsion, but reported a fall after repeated administrations.

No effect of electroconvulsive shock has been found on norepinephrine in the brain which could lead to a persistent increase in the physiologically active concentration of that amine in the brain or to a plausible explanation of the sustained clinical improvement which is usually observed.

In recent studies, we have observed that rats subjected to intermittent electric shock to their feet show an increased rate of disappearance of exogenous tritiated norepinephrine from the brain with little or no decrease in the endogenous levels of that amine. This finding of an increased turnover rate permitted the interpretation that an increased synthesis and utilization of norepinephrine was provoked by such excitation.

If that interpretation was correct and if clinical depression did in fact result to some extent from a deficiency of norepinephrine at certain sites in the brain, it seemed possible that the beneficial effects of repeated electrically induced convulsions lay in a sustained increase in norepinephrine synthesis and utilization in the brain. This hypothesis was tested by examining the turnover rate of norepinephrine in three regions of the central nervous system as measured by the change in specific activity of the labeled amine[12] 24 hours after the termination of a series of electroconvulsive shocks.

Methods.—Forty male albino rats of the Charles River strain, approximately 275 gm in weight, were randomly divided into 4 groups of 10 rats each. Two of these groups were subjected to electroconvulsive shock induced twice daily at 8 A.M. and 4 P.M. for 7 days by transocular application of a single 150-ma shock for 0.2 sec.[13] Following this there was a generalized tonic, then a series of clonic convulsions, followed by postconvulsive coma and stupor from which they recovered within 15 min. The two control groups were treated in exactly the same way except that the switch which applied the current was kept open.

The measurements of norepinephrine levels and turnover were carried out on the day following the last of such shocks; in the intervening 19–24 hr, all animals had been in their cages, provided with food and water ad libitum. All the animals which had received electroshock appeared to behave normally on the morning of the measurements. Between 10:20 A.M. and 11:15 A.M., the 4 groups of rats were injected intracisternally with 20 μl of Merles' solution containing 19.6 μc of 7-H^3 DL norepinephrine (8.8 c/mmole) by a modification[14] of the intraventricular injection technique of Glowinski, Kopin, and Axelrod.[15] This was performed under brief ether anesthesia, and the rats recovered within 10 min of induction. After injection, the rats were returned to their respective cages and left undisturbed until sacrificed by a short stupefying blow and decapitation. Each rat in one control and one experimental group was sacrificed exactly 30 min after the intracisternal injection, and in the remaining 2 groups, 5 hr after the injection.

The brain and spinal cord were quickly removed and the brain was dissected in the cold by removing and discarding the cerebellum, then dividing the remaining portion by a cut above the corpora quadrigemina into one portion containing the brain stem and mesencephalon and another containing the diencephalon and telencephalon. These portions were weighed and kept frozen at $-20°C$ until analysis. The frozen tissues were homogenized in 10 ml of ethanol-water (74:16 by volume), adjusted to pH 6.8, and centrifuged. After remaining at $-20°C$ overnight, the samples were recentrifuged, the supernatant portion diluted with an equal volume of water containing 0.2% ethylenediaminetetraacetate (EDTA) and 0.2% sodium metabisulfite, and passed over an Amberlite CG 50 column buffered at pH 6.1 which retained norepinephrine and normetanephrine.[16] The column was eluted with 5 ml of 0.2 *N* acetic acid, a 2-ml aliquot was taken for the separation of H^3 norepinephrine from H^3 normetanephrine by the alumina adsorption technique of Whitby and collaborators,[17] and the H^3 norepinephrine was assayed by liquid scintillation counting. The endogenous norepinephrine was measured directly on an aliquot of the acidic eluate of the Amberlite column by the spectrofluorimetric method of Euler and Lishajko.[18] Serotonin was measured spectrofluorimetrically by the technique of Snyder, Axelrod, and Zweig.[19] The tissues of all four groups were handled together during the analysis, and corrections were made for recovery of H^3 norepinephrine (70%) and the endogenous amine (70%).

Results.—The results are presented in Table 1. An incidental and unexpected finding was a slight (4.5%) but significant ($p < 0.01$) increase in weight in the experimental group by the end of the electroshock series.

The measurements of endogenous and of H^3 norepinephrine reveal some remarkable changes in the three regions of the central nervous system of the rats exposed to the regimen of electroconvulsive shocks, even though these animals were examined nearly 24 hours after the last shock and at a time when their behavior was not perceptibly different from normal.

In the three regions examined (brain stem and mesencephalon, spinal cord, telencephalon, and diencephalon), the H^3 norepinephrine remaining five hours after its introduction was considerably less in the experimental animals than in their controls. Since the levels at 0.5 hour following injection were not significantly different in the two groups, the rate of disappearance of this material was considerably enhanced in the experimental group. Evidence has been adduced[4, 12] that exogenous radioactive norepinephrine so introduced rapidly labels the endogenous stores in the brain quite specifically, declining at a rate which is an indicator of the turnover of endogenous norepinephrine. From the more rapid fall in specific activity which occurred in the three regions of the experimental animals, an increased turnover, i.e., an increase both in the synthesis and utilization of norepinephrine, can be inferred. The increase in endogenous norepinephrine concentrations, which occurred fairly consistently throughout, further supports the inference of an increase in the synthesis of norepinephrine in these regions which had been sustained for at least 24 hours following the last of a series of electroconvulsive shocks.

If one assumes a single compartmental model for the distribution of norepinephrine in these gross regions of the brain, one may calculate a turnover constant and a rate of turnover for each region of the experimental and control groups (Table 2) indicating an enhancement of synthesis and utilization of 39–57 per cent induced by the experience of repeated electroconvulsive shocks. This simple model, however, is hardly tenable for a complex system like the brain, and in view of the problems inherent in deriving average turnover rates from an over-all examination of a multiexponential system,[20] it seems preferable to draw only the qualitative inference of increased turnover from the data of Table 1. Although even this inference could be incorrect if the electroshock regimen had profoundly altered the size or relationship of the various norepinephrine pools, there is nothing to suggest this possibility, which, even if it occurred, would represent a major effect of electroshock on the disposition of this amine.

The control values for endogenous norepinephrine show a significant fall in each of the three regions examined between the 0.5-hour group (sacrificed in the morning) and the 5-hour group (sacrificed in the afternoon). This may be a reflection of a diurnal variation in norepinephrine levels of the central nervous system.

Dahlström and Fuxe[21] have demonstrated that the cell bodies of the neurons which contain norepinephrine are concentrated in the brain stem, whereas the remainder of the brain and spinal cord appears to contain the axons and axonal endings of such neurons. Our results show that the increased turnover of norepinephrine induced by the electroshock regimen occurs in structures containing cell bodies and structures containing only endings; it appears to be even greater in the latter.

These results appear to confirm the hypothesis which the experiment was designed to test: that repeated electroconvulsive shocks induce a sustained increase in the synthesis and utilization of norepinephrine in the brain. It is of interest that

TABLE 1

EFFECTS OF A COURSE OF ELECTROCONVULSIVE SHOCKS ON ENDOGENOUS AND LABELED NOREPINEPHRINE CONCENTRATIONS IN THE CENTRAL NERVOUS SYSTEM

Regions	Time* (hr)	H^3NE (mμc/gm) C	H^3NE (mμc/gm) E	H^3NE (mμc/gm) Δ %	H^3NE (mμc/gm) p	NE (ng/gm) C	NE (ng/gm) E	NE (ng/gm) Δ %	NE (ng/gm) p	Specific Activity (mμc/μg) C	Specific Activity (mμc/μg) E	Specific Activity (mμc/μg) Δ %	Specific Activity (mμc/μg) p
Brainstem and mesencephalon	0.5	2225 ± 97	2111 ± 74	−5		453 ± 17	589 ± 11	+30	<0.001	4952 ± 317	3595 ± 176	−27	<0.01
	5.0	517 ± 27	380 ± 26	−27	<0.01	356 ± 9	423 ± 18	+19	<0.01	1453 ± 93	914 ± 89	−37	<0.001
Telencephalon and diencephalon	0.5	882 ± 53	984 ± 81	+12		276 ± 9	273 ± 10	−1		3193 ± 224	3616 ± 320	+13	
	5.0	278 ± 24	237 ± 19	−15		237 ± 6	286 ± 15	+21	<0.02	1184 ± 115	851 ± 89	−28	<0.05
Spinal cord	0.5	863 ± 68	940 ± 65	+9		228 ± 4	228 ± 11	0		3776 ± 352	4201 ± 501	+11	
	5.0	125 ± 10	91 ± 8	−28	<0.02	176 ± 6	215 ± 6	+22	<0.001	710 ± 44	425 ± 32	−40	<0.001

Values are the means and standard errors of 9 or 10 animals.
* Time after H^3NE injection.
C = Control rats.
E = Rats subjected to electroconvulsive shock regimen.
Δ = Change (%) from control value.
p Refers to the significance of the difference between C and E by Student's t test in those instances where $p < 0.05$.

TABLE 2

TURNOVER CONSTANTS AND ESTIMATED RATES OF TURNOVER

Regions		K (hr^{-1})	Mean NE (ng/gm)	Rate of NE Turnover (ng/gm hr)	Rate of NE Turnover (Δ % E-C)
Brainstem and mesencephalon	C	0.27	405	109	
	E	0.30	506	152	+39
Telencephalon and diencephalon	C	0.22	257	57	
	E	0.32	280	90	+57
Spinal cord	C	0.37	202	75	
	E	0.51	222	113	+51

Based upon the data of Table 1 and a simple one-compartment model.

this change in the regulation of norepinephrine in the central nervous system persists for at least 24 hours after the last intervention; yet, at that time, is of the same magnitude as the changes we have observed to occur under the immediate influence of drugs or physical stress.

These results may also help to explain certain persistent changes in behavior which others have found following electroshock. Stein[22] reported an experiment in which a persistent increase in the rate of self-stimulation was sometimes observed in rats beginning after 4 days of electroconvulsive shocks twice daily and reaching a maximum after 14 shocks had been administered. Among a large number of drugs examined for their effect on this phenomenon, the same author has observed the most definite effects from those which also act on norepinephrine disposition or metabolism in the brain.[23]

Cohen and Dement[24] reported that paradoxical sleep in cats was reduced significantly following electroconvulsive shock and suggested that a portion of the daily requirement for this type of sleep was satisfied by the convulsive experience. Recently, Pujol and collaborators in this laboratory[25] found a marked increase in the turnover of norepinephrine during paradoxical sleep in regions of the rat brain similar to those in which the present findings indicate an increased norepinephrine turnover induced by electroconvulsive shock. It is possible that electroconvulsive shock can substitute for paradoxical sleep because both provide an augmentation in functionally available norepinephrine.

There is evidence that norepinephrine may not be the only biogenic amine of the brain affected by electroshock. Garratini and Valzelli[26] have previously reported increases in brain serotonin levels following electroconvulsive shock which other workers[27, 28] were unable to confirm. Although we did not measure the turnover of serotonin in the present experiments, determination of endogenous serotonin concentrations gave some indication of an increase in this amine in the brain but not in the spinal cord (Table 3). These changes were not as marked as were the changes in endogenous norepinephrine.

TABLE 3

CONCENTRATION OF ENDOGENOUS SEROTONIN IN THREE REGIONS OF THE CENTRAL NERVOUS SYSTEM

Region	Time (hr)	Endogenous Serotonin Ng/gm C	Endogenous Serotonin Ng/gm E	Δ % E-C	p
Brainstem and mesencephalon	0.5	900 ± 40	1170 ± 30	+29	<0.001
	5.0	1010 ± 40	1110 ± 60	+10	
Telencephalon and diencephalon	0.5	730 ± 20	820 ± 20	+12	<0.01
	5.0	740 ± 20	810 ± 20	+10	<0.05
Spinal cord	0.5	1150 ± 30	1250 ± 60	+9	
	5.0	1210 ± 40	1280 ± 40	+6	

Mean values and standard errors of 8 to 10 animals.

It is hardly likely that a complex psychological state like mood would be modulated by a single chemical substance, and evidence for the involvement of one does not preclude the operation of other neurochemical, neurophysiological, or psychological factors.[29] The finding, however, that electroconvulsive shock, like the drugs which ameliorate depression, has a significant effect on norepinephrine in certain regions of the brain lends credibility to the concept that clinical depression involves

an inadequacy of central norepinephrine mechanisms and that the therapeutic efficacy of electroconvulsive shock may to some extent reside in its ability to stimulate the synthesis and utilization of this amine in the brain.

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