because the lines appear to be narrower at the lower field.

We have determined the absolute sensitivity available from the TMR-32 spectrometer for a phantom sample composed of 4 ml of lean hamburger meat mixed with 150 m*M* KCl to which 12 mg of 90 percent D-[1-^{13}C]glucose had been added so that the labeled glucose concentration in the "tissue" was 15 m*M*. After optimization of pulse width for an interpulse delay of 1.1 seconds and optimal digital filtering, a signal-to-noise ratio of 20:1 was obtained in 12 minutes. Extrapolating from this value, one would expect to be able to identify (with a signal-to-noise ratio of 4) a ^{13}C-labeled metabolite having a concentration of 3 m*M* in 4 ml of tissue in 12 minutes. Furthermore, the 20-cm bore of the 1.89-T magnet makes it possible to perform experiments on the larger organs of larger animals such as cats, and, in general, the sensitivity is expected to increase with the organ volume because the filling factor, which is quite small in the present experiments, can be increased appreciably. The filling factor can also in some cases be improved if minor surgery is performed to place the coil as close as possible to the organ of interest. However, such procedures are often not desirable. Hence, there will be no difficulty in observing in vivo the more abundant metabolites by ^{13}C NMR, provided that the pools can be fully labeled with ^{13}C.

Despite the similarity of cellular suspensions and perfused organs to in vivo conditions, there may be significant differences between the quantitative metabolic fluxes measured by ^{13}C NMR experiments in cells and perfused organs and in vivo conditions, particularly when there are physiological interactions such as those modulated by hormones. We have shown that the major carbohydrate storage pathway of the rat liver is accessible to ^{13}C NMR studies in vivo where previously it could be studied in a perfused organ. Experiments of this type are needed if we are to better understand the physiological control of this pathway in vivo.

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8 June 1981; revised 30 July 1981

Electroconvulsive Shock Increases Tyrosine Hydroxylase Activity in the Brain and Adrenal Gland of the Rat

Abstract. *A single application of electroconvulsive shock produced a rapid but short-lasting increase in tyrosine hydroxylase activity above control values in the rat adrenal medulla and striatum. After repeated electroconvulsive shock treatment (once per day for 7 days), tyrosine hydroxylase activity increased significantly in the locus ceruleus, nucleus of the tractus solitarius, hippocampus, cerebellum, and frontal cortex and remained elevated for 4 to 8 days. Adrenal tyrosine hydroxylase activity increased 1 day after the termination of repeated electroconvulsive shock treatments and remained elevated for at least 24 days, possibly reflecting the establishment of a new and higher steady-state level of catecholamine biosynthesis in the adrenal. These findings suggest that the persistent changes in tyrosine hydroxylase activity produced by repeated electroconvulsive shock may be a factor contributing to the long-lasting antidepressant effects of this treatment.*

Electroconvulsive shock (ECS) is the most effective therapy available for the treatment of endogenous depression (*1*), although the precise mechanism underlying the antidepressant effects of ECS remains unclarified. Understanding the antidepressant mechanism of ECS would not only aid in the development of more effective treatments for depression but also might provide insight into the etiology of depression. In order to elucidate the biochemical mechanism of the antidepressant effects of ECS, two crucial factors should be taken into account: (i) the antidepressant effects of ECS appear only after repeated treatments, and (ii) the antidepressant effects of ECS persist for an extended period of time after the termination of treatment. It therefore appears likely that any biochemical change that might explain the mechanism underlying the antidepressant effect of ECS should also persist for a period of time after the termination of a series of ECS treatments (*2*).

A variety of biochemical changes occur in the central nervous system (CNS) after repeated ECS treatment that are not apparent after a single ECS treatment, including an increase in norepinephrine turnover (*3*, *4*), an increase in norepinephrine tissue concentrations (*3*), a decreased affinity for norepinephrine of the high-affinity neuronal uptake system (*5*), a decrease in postsynaptic beta receptor density (*6*), and a decrease in the activity of the norepinephrine-stimulated adenosine 3′,5′-monophosphate

0036-8075/81/1106-0662$01.00/0

Table 1. The effects of seven electroconvulsive shocks (one per day) on brain and adrenal gland TH activity assayed from nonshocked animals (sham controls) and from animals killed at 1, 4, 8, 16, and 24 days after the last shock. The results (in nanomoles per hour per milligram) are the means of six experiments ± the standard error of the mean. All values were determined at 0.1 m*M* L-[l-^{14}C]tyrosine and 0.25 m*M* 6-MePtH$_4$.

Location	Control	Days after repeated ECS treatment				
		1	4	8	16	24
Adrenal gland	20.9 ± 1.51	36.3* ± 2.36	36.3* ± 1.15	30.1† ± 3.14	26.9† ± 2.30	27.1† ± 2.40
Substantia nigra	5.58 ± 0.35	5.24 ± 0.31	5.39 ± 0.38	5.72 ± 0.42	5.54 ± 0.43	5.79 ± 0.39
Striatum	8.03 ± 0.39	8.20 ± 0.42	8.52 ± 0.49	7.87 ± 0.38	8.42 ± 0.45	8.14 ± 0.41
Locus ceruleus	2.03 ± 0.23	4.21* ± 0.33	3.40* ± 0.35	2.15 ± 0.13	2.14 ± 0.21	1.98 ± 0.30
Nucleus of the tractus solitarius	1.61 ± 0.22	2.19† ± 0.23	2.64* ± 0.11	1.73 ± 0.24	1.60 ± 0.23	1.53 ± 0.29
Cerebellum	0.26 ± 0.03	0.29 ± 0.02	0.39* ± 0.02	0.38* ± 0.03	0.36 ± 0.04	0.30 ± 0.03
Frontal cortex	0.29 ± 0.03	0.30 ± 0.02	0.51* ± 0.02	0.46* ± 0.05	0.38 ± 0.06	0.33 ± 0.02
Hippocampus	0.22 ± 0.03	0.16 ± 0.02	0.34* ± 0.02	0.27 ± 0.04	0.27 ± 0.03	0.24 ± 0.04
Hypothalamus	4.30 ± 0.47	4.38 ± 0.35	4.57 ± 0.19	4.69 ± 0.43	4.64 ± 0.50	5.06 ± 0.51

*Significantly different from control; $P < .01$. †Significantly different from control; $P < .05$.

generating system (*7*). Since these changes are apparent only after repeated ECS treatment, it is likely that the antidepressant effects of ECS may be associated with a catecholaminergic mechanism. However, the extent to which these changes persist in the brain and the specificity of these changes to discrete brain regions have not been systematically assessed. In order to evaluate more thoroughly the effects of repeated ECS on catecholaminergic neurons, we measured the activity of tyrosine hydroxylase (TH) in eight brain regions and the adrenal gland after a series of seven ECS treatments. Tyrosine hydroxylase is of special interest since it is thought to be the rate-limiting enzyme in the biosynthesis of catecholamines (*8*) and is known to be activated (*9–11*) or induced (*12, 13*) after a number of treatments.

Musacchio *et al.* (*14*) reported a slight increase in the activity of TH prepared from brainstem, mesencephalon, and cortex after repeated ECS. However, in their study TH activity in more discrete brain regions and the persistence of the effect were not examined. In the present study, we have determined that repeated ECS treatment produces an increase in TH activity within specific brain regions including the locus ceruleus, nucleus of the tractus solitarius, hippocampus, frontal cortex, and cerebellum, as well as in the adrenal medulla. These changes persist for 4 to 24 days after the last ECS treatment.

The ECS was administered to male Sprague-Dawley rats by the application of 300 mA of current transorbitally for a duration of 0.2 second. Either 5 or 60 minutes after the single ECS treatment, the rats were anesthetized with pentobarbital (60 mg per kilogram of body weight), and the adrenal glands were removed surgically to avoid the activation of adrenal TH which ordinarily occurs as a result of decapitation (*11*). The animals were then decapitated, and brain areas were dissected out; tissues were weighed and homogenized in ten volumes (adrenal, striatum), eight volumes (substantia nigra), six volumes (hypothalamus), five volumes (locus ceruleus, nucleus of the tractus solitarius, cerebellum), four volumes (frontal cortex), and three volumes (hippocampus) of 50 m*M* tris-acetate buffer, *p*H 6, containing 0.2 percent Triton X-100. The homogenates were centrifuged at 40,000*g* for 30 minutes at 4°C, and 10 μl of the supernatant was assayed for TH activity. The TH activity was determined by the coupled decarboxylase assay as modified in our laboratory (*15*). We studied the effects of repeated ECS on brain and adrenal TH activity by administering ECS once daily for 7 days and assaying for TH activity in the adrenal and the eight brain regions at 1, 4, 8, 16, and 24 days after the last ECS treatment.

Five minutes after the single application of ECS, TH activity was significantly increased above control value in both the adrenal and striatum (Fig. 1); TH activity in the other brain regions was unaffected. The acute activation of adrenal and striatal TH by ECS was the consequence of the conversion of the enzyme from a form with a low affinity for pterin cofactor to a form with a high affinity for cofactor. In the unstressed rat, adrenal TH activity exhibits curvilinear kinetics when analyzed by the method of Lineweaver and Burk. This nonlinear relationship suggests that substantial amounts of both high- and low-affinity forms of the enzyme are present in the adrenal (*11, 16*). The single application of ECS appears to increase the linearity of the Lineweaver-Burk plot for adrenal TH activity, an indication that this treatment produces a conversion of the low-affinity form of the enzyme to the high-affinity form. However, the conversion

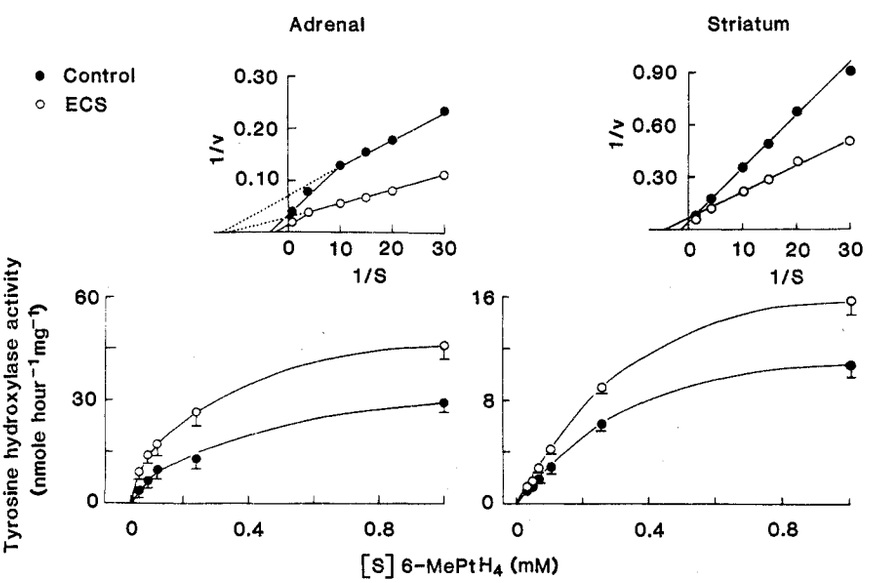

Fig. 1. Kinetic analysis of the effects of a single ECS (300 mA for 0.2 second) on adrenal and striatal TH activity at various cofactor (6-MePtH$_4$) concentrations. The results are means from six experiments ± the standard error of the mean. All values were determined at 0.1 m*M* L-[1-^{14}C]tyrosine.

does not appear to be complete since nonlinearity still exists at the highest cofactor concentration examined (1.0 m*M*).

The activation of TH in the striatum by ECS is accompanied by a shift in the K_m value (Michaelis constant) for 6-methyl-5,6,7,8-tetrahydropterin (6-MePtH_4) from 0.67 to 0.22 m*M*. Striatal TH from both unstressed and ECS-treated rats does not exhibit nonlinear kinetics when the reciprocal of the enzyme velocity (v) is plotted against the reciprocal of the 6-MePtH_4 concentration (S). This result suggests that the activity of striatal TH obtained from the nonshocked animal is mainly or entirely in the low-affinity form and that, after ECS treatment, striatal TH is converted almost completely or entirely to the high-affinity form. Sixty minutes after a single application of ECS, adrenal and striatal TH activity had returned to control values.

Various stresses (*13*) and drug treatments (*12*) can produce an increase in TH activity in the adrenal and locus ceruleus that does not become apparent until 12 to 24 hours after the treatment. In order to determine whether a single application of ECS can also produce a delayed increase in TH activity, we examined TH activity in the adrenal and the eight brain regions 24 hours after ECS. No change in TH activity was found.

The activation of TH after a single administration of ECS is rapid and of brief duration and thus is not temporally correlated with the long-term antidepressant effects of ECS treatment. Activation of TH similar to that seen in the adrenal and striatum after a single application of ECS occurs in the hypogastric nerve–vas deferens preparation after acute nerve stimulation (*9*), in the adrenal after decapitation stress (*11*), and in the striatum after short-term treatment with antipsychotic drugs (*10*).

Twenty-four hours after the application of ECS daily for 7 days, TH activity was significantly increased in the adrenal gland, locus ceruleus, and nucleus of the tractus solitarius (Table 1). In order to evaluate whether the changes in enzyme activity were due to an increase in enzyme protein, we carried out immunotitration studies on homogenates of the adrenal, locus ceruleus, and nucleus of the tractus solitarius 24 hours after the last ECS treatment (*17*). Calculations made from the immunotitration curves indicate that TH enzyme protein in homogenates obtained from ECS-treated animals increased 77 percent in the adrenal, 111 percent in the locus ceruleus, and 38 percent in the nucleus of the tractus solitarius as compared with tissues prepared from nonshocked animals. These increases in TH enzyme protein are similar to the percentage increases in TH activity obtained when these tissues are assayed 24 hours after the last of the sequences of ECS treatments (Table 1). Four days after the termination of repeated ECS, TH activity remained elevated in the adrenal, locus ceruleus, and nucleus of the tractus solitarius; in addition, TH activity increased in the hippocampus, cerebellum, and frontal cortex. The activity of the enzyme in the cerebellum and frontal cortex remained elevated for an additional 4 days (Table 1). The noradrenergic cell bodies of the locus ceruleus send projections to numerous brain areas, including the cerebellum, hippocampus, and frontal cortex (*18*). On the basis of estimates of the rate of TH transport in central noradrenergic neurons [approximately 2 to 7 mm per day (*19*)], these data suggest that the increase in TH enzyme protein produced in the cell bodies of the locus ceruleus after repeated ECS is eventually transported to the nerve terminal regions: hippocampus, cerebellum, and frontal cortex. After repeated ECS, no change in TH activity is found in the substantia nigra, striatum, or hypothalamus (Table 1), an indication that the increase in TH activity in the CNS in response to repeated ECS is limited to specific brain areas, is not a generalized response of all catecholaminergic neurons within the brain to this treatment, and may be restricted to noradrenergic neurons.

A 74 percent increase in adrenal TH activity occurred at 1 and 4 days after repeated ECS, and TH activity remained elevated approximately 44 percent at 8 days and approximately 30 percent at 16 and 24 days. If the half-life of adrenal TH is estimated to be 3 days (*20*), this persistent elevation of TH activity in the adrenal for at least 24 days after repeated ECS may reflect the establishment of a new and higher steady-state level of catecholamine biosynthesis in the adrenal.

The changes in TH activity in the brain that are apparent for at least 8 days after repeated ECS may be responsible for, or contribute to, the antidepressant effect of this treatment. This does not exclude the possibility that other changes that occur in the brain after repeated ECS might also play a part in this antidepressant action, such as increases in metenkephalin in the septum and hypothalamus that persist for 6 days after repeated ECS (*21*) or changes in monoamine oxidase that occur in whole brain after repeated ECS and persist for 19 days (*22*). It is tempting to speculate that the more prolonged change in adrenal TH activity after repeated ECS might contribute to the persistence of the antidepressant effect, even at times when changes in TH activity in the CNS are no longer apparent. In depressed patients, the initial application of ECS produces a rapid increase in plasma epinephrine concentrations which returns to control values within 10 minutes (*23*). Havens *et al.* (*24*) reported that, after repeated ECS (five to nine treatments), an enhancement occurs in both the preseizure and the postseizure plasma epinephrine concentrations of patients as compared with the plasma epinephrine concentrations in the same patients before and after the initial application of ECS.

The interrelationships among our findings of an increase in TH activity in the adrenal after repeated ECS, the effect of repeated ECS on the adrenal content of catecholamines, the release of these catecholamines into the blood, and the responsiveness of the adrenal medulla to stress require further investigation to ascertain more definitively the functional significance of these changes in the adrenal medulla after repeated ECS.

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25. This work was supported by PHS grants NS 07927 and NS 09199.

11 June 1981

Polysaccharides in Soil Fabrics

Abstract. *Treatment of ultrathin sections of natural soil fabrics with heavy metal stains, specific for carbohydrates, showed that polysaccharides are widely distributed in soils. In addition to being associated with living cells and dead remains of plants and microbes, carbohydrates also coat clay platelets and occur in crevices of submicron size within mineral aggregates. The determination of the precise location of polysaccharides in soils explains in part why some carbohydrates are resistant to microbial degradation and why small quantities of microbial polysaccharides are able to stabilize clay aggregates.*

Between 50 and 80 percent of the dry weight of plants consists of carbohydrates (*1*), the most abundant organic materials entering soils (*1, 2*). Carbohydrates constitute between 5 and 10 percent of the soil organic matter (*3*), and they are important in the maintenance of aggregate stability, in the ion-exchange properties of soils, and in the nutrition of heterotrophic soil microorganisms (*2, 3*). Although chemical analyses of carbohydrates extracted from soils have provided qualitative and quantitative data on the total amount of carbohydrates (*1, 2*) and on the relative proportions of individual monosaccharides in soils (*1*), the precise location of most carbohydrate in situ in natural soil fabrics is unknown.

With the solution of the technical problems arising from the preparation of ultrathin sections of soils, it is possible to locate soil components in situ, provided that the components are electron-opaque (*4*). Soil minerals have a natural intrinsic electron density, and many organic components associated with living plant, animal, and microbial cells can be made electron-dense if one modifies the techniques commonly used in the preparation of biological tissues for electron microscopy (*4–6*). Using these methods, one can identify fragments of decomposing tissues down to submicron sizes (*4*) and recognize humified amorphous materials almost to their macromolecular dimensions. Unfortunately, however, many carbohydrates do not react with the fixatives and heavy metal stains normally used in electron microscopy (*4, 6, 7*). Thus the location of carbohydrates in ultrathin sections of soil fabrics has been inferred on morphological rather than histochemical grounds. Direct localization of polysaccharides in situ in undisturbed soil fabrics has largely been restricted to those materials such as root or microbial mucilages whose position in the fabric is already known (*4–6*) and whose slight electron density may be attributable to the traces of noncarbohydrate materials such as proteins and polyphenols that they contain (*7*). The presence of carbohydrates near biological objects can sometimes be inferred from a lack of electron density in an otherwise electron-opaque fabric. For example, in soil fabrics containing microorganisms (Fig. 1) there is usually an electron-transparent space surrounding bacteria into which the clay platelets do not penetrate. Since soil organisms are known to secrete carbohydrate slimes and capsule materials, it is assumed that the apparent "void" is filled with electron-transparent microbial polysaccharide, but the nature of the majority of electron-transparent spaces of submicron size (for example, *V* in Fig. 1) cannot be determined with the same confidence. Roots that secrete carbohydrate gels may occupy less than 1 percent of the bulk soil volume, and microbial cells constitute only a small percentage of the soil biomass (*8*). Thus, the polysaccharide associated with living cells does not account for all the carbohydrate found as a result of chemical analysis of soils. It is therefore of particular importance to locate carbohydrate in situ in natural soil fabrics by positive and specific histochemical reactions.

An extensive literature has accumulated on methods for positively staining polysaccharides at the ultrastructural level (*9–12*). Of these, the ruthenium red/OsO_4 (Ru/Os) stain for acidic polysaccharides and the silver methenamine and silver proteinate methods for carbohydrates reactive to periodic acid–Schiff bases (PA/Ag) have proved to be the most reliable and least controversial (*9, 10*).

The Ru/Os stain of Luft is generally assumed to stain carbohydrates containing polyuronides and has been widely used to demonstrate microbial slimes and capsule materials in natural environments including tissues and soil fabrics (*4*). Figure 2 shows Ru/Os-reactive fibrous materials in a natural soil fabric containing microorganisms. The fibers are about 8 to 10 nm in diameter and of indeterminate length, but they stretch across fine pores of submicron diameter to form an open mesh in which small stacks of clay platelets become entangled. These fibrous networks are commonly observed in crumbs from A horizons of soils under pasture, and the fibrils may persist long after the death of the microorganisms that secreted them (*4*).

Not all Ru/Os-reactive carbohydrates in soils are fibrous, however. Both roots (*5*) and bacteria (*4, 6*) secrete gels that are granular in texture, and small pockets of granular material are often observed in soils in the absence of living cells. These deposits are small but widespread and are found in crevices between stacks of clay platelets (Fig. 3). This means that not only are the fine pores occluded, but that the clay stacks are bound together by the gels.

0036-8075/81/1106-0665$01.00/0