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Entorhinal Cortex Lesions Induce a Decreased Calcium Transport in Hippocampal Mitochondria

Abstract. *Lesions to the entorhinal afferent of the hippocampus in rats caused marked changes in calcium transport into mitochondria. Pyruvate-supported calcium transport into mitochondria from the denervated hippocampus was decreased to a larger extent than succinate-supported transport, and adenosine triphosphate–supported transport was not significantly modified. Although cytochrome oxidase and succinate dehydrogenase activities were not significantly changed by entorhinal lesions, pyruvate flux through pyruvate dehydrogenase was significantly decreased, and this effect was correlated with changes in pyruvate-supported calcium transport. The active portion of pyruvate dehydrogenase decreased, whereas total pyruvate dehydrogenase was not modified. These data suggest that denervation might initiate dendritic atrophy and subsequent growth responses by modifying calcium regulation through a change in the phosphorylation of pyruvate dehydrogenase.*

Partial denervation of the hippocampus sets in motion a series of anatomical changes, including dendritic atrophy (*1*), glial hypertrophy and atrophy (*2*), and a growth response in undamaged fibers ("sprouting") and denervated dendrites (*3*). Because these phenomena are a clear manifestation of anatomical plasticity of mature neuronal circuits and because they may be involved in the behavioral and physiological sequelae of brain damage, it is important to identify the biochemical processes that promote and regulate them. Studies of plasticity resulting from electrical stimulation of hippocampal afferents have suggested the existence of a cellular mechanism that could mediate the degenerative and growth responses to denervation. High-frequency activation of certain hippocampal pathways modifies the endogenous phosphorylation of a protein (*4*) that has been identified as the alpha subunit of the mitochondrial enzyme pyruvate dehydrogenase (PDH) (*5*). Pyruvate dehydrogenase activity, which is regulated by the phosphorylation and dephosphorylation of its alpha subunit (the enzyme being inactive in its phosphorylated form), is, in turn, tightly coupled to calcium sequestration by mitochondria (*6*). These results prompted the hypothesis that the regulation of cytosol calcium in the pre- and postsynaptic elements of neuronal connections is strongly influenced by synaptic activity (*7*). Increases in cytosol calcium levels have been linked both to growth (*8*) and degeneration (*9*) in neurons. Therefore the possibility exists that denervation initiates dendritic atrophy and subsequent growth responses (formation of new spines) by removing presynaptic regulation of the calcium "buffering" mechanism provided by mitochondria in the denervated cells. We therefore measured calcium transport and PDH activity in hippocampal mitochondria of rats after producing lesions of the entorhinal cortex.

One week after unilateral lesioning of the entorhinal cortex, the pyruvate-supported calcium accumulation by the crude mitochondrial fraction was markedly reduced (−41 percent) in the ipsilateral, denervated hippocampus as compared with the contralateral, control hippocampus (Table 1). The values found in the contralateral hippocampus were not significantly different from those in the hippocampi of control, unlesioned rats (*10*). The succinate-supported calcium accumulation was affected to a lesser extent (−25 percent), and the adenosine triphosphate (ATP)–supported calcium accumulation was not significantly altered (Table 1). The ratios of pyruvate to succinate and of pyruvate to ATP were thus markedly reduced on the lesioned side (Table 1). In the same group of animals, metabolic flux through PDH was also significantly reduced (−25 percent) (Table 1). At various times after lesioning, the changes in pyruvate decarboxylation correlated well with the changes in pyruvate-supported calcium transport (Fig. 1); the slope of the curve was about 0.54 and the coefficient of correlation was .83 ($P < .01$). However, there was no correlation between the changes in pyruvate-supported calcium transport and the changes in succinate dehydrogenase (SDH) activity ($r = .19$). Only the active portion of PDH was decreased on the lesioned side (−22 percent), the total PDH activity being unmodified (Table 2). The ratio of active to total PDH activity, which is generally considered an index of the phosphorylated state of PDH (*11*), was therefore also significantly decreased (−20 percent). The decrease in pyruvate-supported calcium transport was present as early as 24 hours after production of the lesion, reached a maximum about 5 days after lesioning, and was still present and of a similar magnitude as long as 6 months

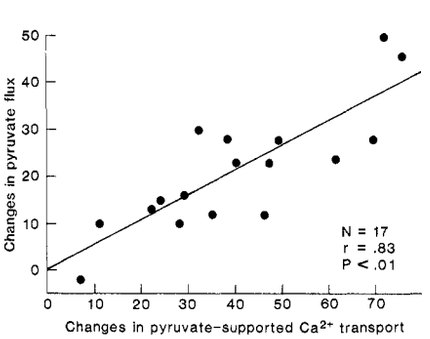

Fig. 1. Correlation between changes in pyruvate-supported calcium transport and changes in pyruvate flux at various times after lesioning of the entorhinal cortex. At various times (1 day to 6 months) after production of a unilateral entorhinal cortex lesion, calcium transport supported by pyruvate and pyruvate flux through pyruvate dehydrogenase were determined in mitochondrial fractions prepared from hippocampus (legend to Table 1). The changes in both parameters represent the percent decrease on the lesioned side in comparison with the control side.

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later. One week after lesioning, the decrease in pyruvate-supported calcium accumulation was similar in free and synaptic mitochondria, thus excluding the possibility that these changes reflect a decreased number of presynaptic mitochondria in the deafferented hippocampus. The relative integrity of hippocampal mitochondria was further assessed by measuring the activity of two other mitochondrial enzymes; SDH activity was slightly decreased 1 week after lesioning, and the activity of cytochrome oxidase was unchanged (Table 1).

These data indicate that deafferentation of the hippocampus, by removal of its major input, markedly reduces the ability of mitochondria to accumulate calcium when the metabolic pathway is fueled with pyruvate. This effect is probably not simply due to a gross alteration of hippocampal mitochondria, since substituting ATP for pyruvate as the energy source reestablishes normal calcium transport, and succinate substitution only partially restores this function. Pyruvate dehydrogenase is the rate-limiting enzyme in oxidative metabolism, at least in brain (*12*), and changes in pyruvate-supported calcium transport are likely to be more significant than changes in either succinate- or ATP-supported transport. These data, together with the fact that the activities of two mitochondrial enzymes are not significantly modified, make it unlikely that a decreased number of mitochondria in the denervated hippocampus is responsible for the observed effects. In view of the correlation between changes in active PDH and changes in pyruvate-supported calcium transport, it seems likely that the locus of the metabolic alteration occurs at the PDH level. This is corroborated by the decrease in active PDH while total PDH activity is not modified. The fact that changes in calcium transport have about twice the amplitude of the changes in pyruvate decarboxylation might reflect the complex relationships between pyruvate flux through PDH and the generation of the proton generative force necessary to carry calcium ions inside the mitochondria (*13*). Although the precise modification of the PDH complex is not clear at the moment, one possibility is that the state of phosphorylation of the α-PDH is modified either by an activation of the pyruvate kinase or an inhibition of the PDH phosphatase (*14*).

It has been shown that denervation results in various metabolic alterations in the target zones. For instance, at the neuromuscular junction, denervation, as well as disuse, induces a dramatic decrease in the levels of mitochondrial enzymes (*15*); lesions of the locus coeruleus are accompanied by a decreased cerebral oxidative metabolism in the "active state" (*16*), whereas lesions of the nigrostriatal dopaminergic neurons result in a decrease in SDH activity in the striatum (*17*). In these cases, it is not clear whether the observed effects are a direct result of denervation or a consequence of decreased functional activity in the denervated regions. Functional activity in the hippocampus is only slightly altered after lesioning of the entorhinal cortex (*18*), suggesting that the changes seen after lesioning probably reflect the loss of a modulatory factor normally provided by the entorhinal afferents. Whether this factor is a neurotransmitter, ions, or peptides is still a matter of speculation.

The decreased ability of mitochondria to take up calcium from the cytoplasm may have important functional consequences in the denervated dendrites. Mitochondria are generally considered to be calcium sinks, and recent studies have shown that these organelles are able not only to take up calcium with an affinity in the low micromolar range (*19*), but also to buffer cytosol calcium at this low concentration (*20*). The reduced capacity of mitochondria to take up calcium after lesioning may cause calcium levels to rise considerably, at least under some circumstances. In agreement with this idea, we have preliminary evidence that the "set point" at which mitochondria buffer free calcium concentrations is

Table 1. Mitochondrial calcium transport (expressed as nanograms per milligram of protein) and enzyme activity (expressed as nanograms per milligram of protein per hour) 1 week after unilateral lesioning of the entorhinal cortex. Male Sprague-Dawley rats (200 to 250 g) were killed by decapitation. The hippocampi were rapidly dissected and homogenized in cold 0.32*M* sucrose, and crude mitochondrial fractions were prepared by differential centrifugation. The pellets were resuspended in buffer containing Hepes (20 m*M*), KCl (150 m*M*), NaH_2PO_4 (2.4 m*M*), $MgSO_4$ (1.3 m*M*), and enough tris-base to bring the *p*H to 6.6. Calcium accumulation supported by pyruvate (0.1 m*M*), succinate (5 m*M*), or ATP (1 m*M*) was measured at a free calcium concentration of 2.0 μ*M* (*6*). This calcium concentration was obtained by the use of a calcium-EGTA buffer [200 μ*M* EGTA and 120 μ*M* ^{45}Ca (ICN, Irvine, California; specific activity, 3.5 Ci/g)]. Cytochrome oxidase and succinic dehydrogenase activities were measured with spectrophotometric assays (*25*). Flux through pyruvate dehydrogenase was determined by measuring the $^{14}CO_2$ produced from [1-^{14}C]pyruvate (Amersham; specific activity, 23.8 mCi/mmole; final concentration, 40 μ*M*) by the mitochondrial preparation (*6*). Entorhinal cortex lesions were produced as in (*1–3*). A paired *t*-test was used in comparing lesioned and control sides. *N*, number of experiments. P/S represents the ratio of pyruvate- to succinate-supported calcium transport, and P/A represents the ratio of pyruvate to ATP-supported calcium transport. Values are means ± S.E.M. N.S., not significant.

Item	*N*	Lesioned side	Control side	Percent change	*P*
		Ca^{2+} transport			
Pyruvate supported	12	291 ± 80	468 ± 92	−41 ± 6	<.001
Succinate supported	12	1461 ± 196	1869 ± 166	−23 ± 9	<.05
ATP supported	11	605 ± 69	649 ± 51	−7 ± 6	N.S.
P/S	12	0.19 ± 0.03	0.27 ± 0.05	−30 ± 5	<.01
P/A	11	0.41 ± 0.06	0.67 ± 0.11	−38 ± 6	<.01
		Enzyme activity			
Cytochrome oxidase	9	11.8 ± 0.9	11.1 ± 1.3	+12 ± 11	N.S.
Succinate dehydrogenase	9	1.01 ± 0.10	1.20 ± 0.11	−12 ± 8	N.S.
Pyruvate flux	4	0.16 ± 0.02	0.21 ± 0.02	−22 ± 3	<.02

Table 2. Effects of unilateral lesions of the entorhinal cortex on active and total pyruvate dehydrogenase activity. After preparation of crude mitochondrial fractions (legend to Table 1), the pellets were resuspended by sonication in cold 25 m*M* 1,4-piperazinediethanesulfonic acid (Pipes) buffer, *p*H 7.2, containing 1 m*M* EDTA. A mixture of EGTA (2 m*M*), dichloroacetate (2.5 m*M*), and sodium fluoride (8 m*M*) was added to a first portion to prevent further PDH kinase and phosphatase activities (*26*). This was considered as representing the active portion of PDH. A second portion was incubated at 30°C for 30 minutes with a mixture of $CaCl_2$ (1.5 m*M*), $MgCl_2$ (10 m*M*), and dinitrophenol (0.1 m*M*) to totally dephosphorylate the alpha subunit of PDH. This was considered as representing total PDH activity. In both cases, PDH activity was measured as the initial rate of conversion of [^{14}C]pyruvate by a modification (*6*) of the method of Leiter *et al.* (*27*). Results, expressed in nanomoles of CO_2 formed per minute per milligram of protein, are means ± S.E.M. of six experiments.

Side	*N*	Active	Total	Active/total (%)
Lesioned	6	4.37 ± 0.21*	11.2 ± 0.5	39 ± 2*
Control	6	5.59 ± 0.33	11.4 ± 0.4	49 ± 2

*$P < .01$ by paired *t*-test.

elevated on the lesioned side as compared to the control side when mitochondria are fueled with pyruvate (*21*). This would result in the activation of various calcium-dependent mechanisms and of proteolytic processes that could induce the dissolution of axoplasm and disruption of the cytoskeleton (*22*), as has been proposed for degenerative changes in peripheral neurons (*9*). The activation of these proteolytic processes might be responsible for the initial atrophy in the denervated dendrites, as well as the subsequent generation of appropriate sites for reinnervation (*1–3*).

Our results suggest that different forms of plasticity in the hippocampus might be initiated by similar biochemical mechanisms. The modification of the state of phosphorylation of α-PDH by high-frequency stimulation (*4*) or learning (*11*) and by hippocampal denervation might result in a decreased ability of mitochondria to rapidly eliminate excess cytosolic calcium, followed by an activation of calcium-sensitive proteolytic processes. In plasticity induced by high-frequency stimulation or learning, this activation could be limited in time and space and result in local membrane changes that cause the unmasking of neurotransmitter receptors (*23*) or a change in spine shape (*24*). With denervation, this activation would be more dramatic in extent and longer lasting, leading initially to a resorption of dendritic spines and later to reactive synaptogenesis. This raises the possibility that plasticity reflects the potential ability of synaptic connections to evolve from stability to instability to irreversible instability and degeneration.

MICHEL BAUDRY
JANNON FUCHS
MARKUS KESSLER
DENISE ARST
GARY LYNCH

Department of Psychobiology, University of California, Irvine 92717

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Cholinergic Innervation in Neuritic Plaques

Abstract. *Although several studies of Alzheimer's disease suggest that the frequency of neuritic plaques in the cerebral cortex is correlated with the severity of dementia and with reduction in presynaptic cholinergic markers in the cortex, the relationship between cholinergic cortical innervation and the pathogenesis of plaques is unknown. The hypothesis was tested that the neurites in the plaque consist, in part, of presynaptic cholinergic axons, many of which arise from neurons in the basal forebrain. This hypothesis was tested by analyzing the character and distribution of plaques in monkeys, aged 4 to 31 years, with staining for acetylcholinesterase and also with Congo red and silver stains. Immature and mature plaques were rich in acetylcholinesterase. As the plaques matured, the amount of amyloid increased, and the number of neurites and the activity of acetylcholinesterase decreased. End-stage amyloid-rich plaques lacked acetylcholinesterase. These observations indicate that changes in cortical cholinergic innervation are an important feature in the pathogenesis and evolution of the neuritic plaque.*

Alzheimer's disease (AD) and senile dementia of the Alzheimer's type (SDAT) are the most common causes of dementia in middle and late life (*1, 2*). The principal histological hallmarks of the disease are neurofibrillary tangles and neuritic plaques. The latter are composed of degenerating axonal terminals, amyloid, and reactive cells (*1, 3, 4*). Although not all reports agree (*5*), in studies utilizing systematic testing, patients with severe dementia showed the greatest number of neuritic plaques (*6*) and the most profound loss of the cortical cholinergic presynaptic markers, acetylcholinesterase (AChE) and choline acetyltransferase (CAT) (*7, 8*).

Our previous studies of AD/SDAT (*9*) have shown a severe loss of neurons in the nucleus basalis of Meynert (nbM), a nucleus of the basal forebrain (*10*) that projects directly and diffusely to neocortex (*11*). Neurons in the nbM provide the major source of cortical cholinergic innervation. The nbM shows high levels of CAT activity, and its large neurons are rich in AChE and stain with antibodies directed against CAT (*12–15*); undercutting the cortex or selective destruction of the neurons in the nbM is associated with reductions in cortical AChE and CAT levels (*13, 15*). These lines of evidence, in conjunction with the observation that some plaques in patients with AD/SDAT contain AChE (*16*), suggest that some of the dystrophic neurites in immature and evolving plaques may be degenerating intracortical branches of nbM axons. If this hypothesis is correct, the neurite-containing plaques

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