## Noradrenaline and Inhibition of Renshaw Cells

Abstract. *Although postsynaptic inhibition of Renshaw cells in the spine is blocked by strychnine the depression of these neurons by electrophoretically administered noradrenaline is resistant to this alkaloid. This finding raises doubts as to whether noradrenaline is an inhibitory transmitter in the spinal cord of the cat.*

The histochemical detection of noradrenaline within nerve fibers and terminals of the spinal cord (*1*), together with the effects of systemically administered L-3,4-dihydroxyphenylalanine on the spinal reflexes of unanesthetized cats (*2*) has given rise to speculation concerning the possible function of noradrenaline as a synaptic transmitter. Although electrophoretically administered noradrenaline has been reported not to excite spinal interneurons or to depress the synaptic responses of these cells in cats anesthetized with pentobarbitone (*3*), the finding that this monoamine has a depressant action on cortical and thalamic neurons (*4*) has led to reinvestigation of its action on spinal neurons. Noradrenaline, when administered electrophoretically to unanesthetized cats, depresses the synaptic responses of spinal interneurons and also reduces the sensitivity of these and Renshaw cells to DL-homocysteic acid (*5*). Renshaw cells are readily inhibited by volleys reaching the spinal cord via dorsal roots (*6*) and from supraspinal centers (*7*). Hence, we compared noradrenaline-induced depression of Renshaw cells with such inhibition, particularly as the inhibition of Renshaw cells has been reported to be sensitive to strychnine (*6*).

All of the experiments were carried out in unanesthetized *cerveau isolé* cats, the brainstem having been transected at the mid-collicular level by radio-frequency coagulation under halothane anesthesia. The animals were paralyzed with gallamine triethiodide and artificially respired. Extracellular action potentials of single spinal interneurons and of Renshaw cells were recorded by means of the 4*M*-NaCl-containing center barrel of five-barrel electrodes (overall tip diameter 4 to 6 $\mu$), and the drugs were administered electrophoretically from aqueous solutions contained in the other barrels (*8*). Interneurons of the seventh lumbar segment were identified by the repetitive synaptic response to stimulation of ipsilateral hind limb nerves or the segmental dorsal root; Renshaw cells were identified by the typical response to antidromic stimulation of the ventral root.

Both the spontaneous discharge and the firing of interneurons induced by DL-homocysteic acid were depressed by noradrenaline ejected with currents of 10 to 40 na from a 0.1*M* solution of noradrenaline bitartrate. Similar effects

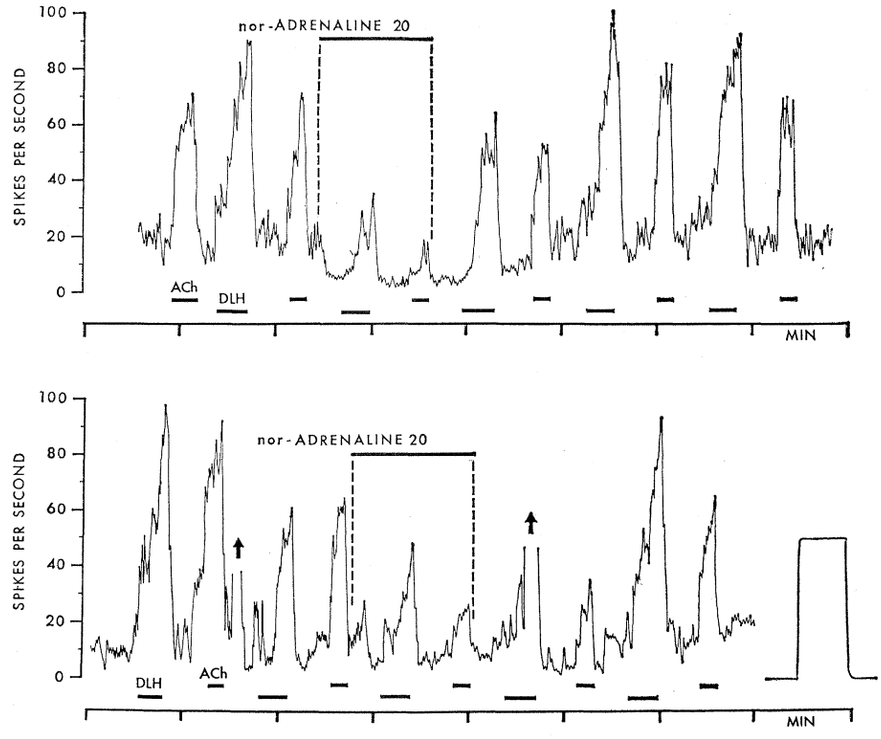


Fig. 1. Effect of noradrenaline (20 na) upon the sensitivity of a Renshaw cell to electrophoretically administered acetylcholine (ACh, 3 na) and DL-homocysteic acid (DLH, 11 na) before (top) and 3 minutes after (bottom) intravenous administration of strychnine hydrochloride (0.05 mg/kg). Electrophoretic ejections are indicated by the horizontal black lines. The arrows mark "spontaneous" bursts of high-frequency firing. (Bottom, right) The final tracing shows the response of the frequency recording system to a 50-cy/sec test signal of 30-second duration.

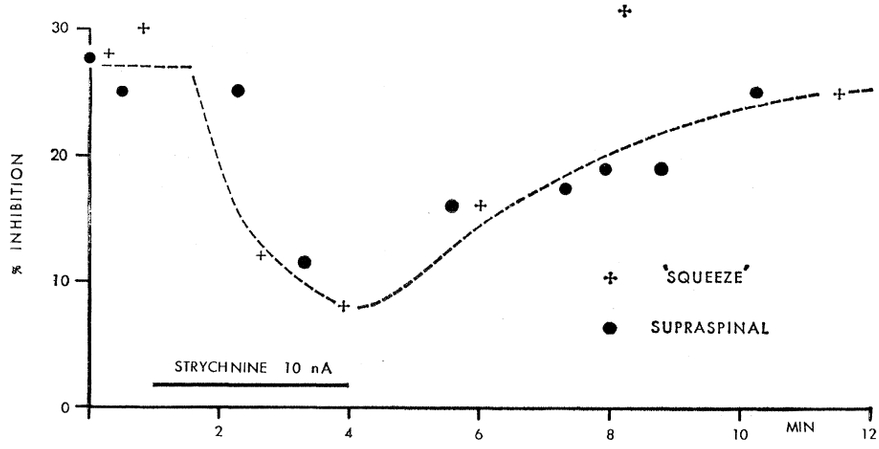


Fig. 2. Effect of electrophoretically administered strychnine (10 na) upon the inhibition of a Renshaw cell produced by squeezing the ipsilateral hind limb (+) and by electrical stimulation (seven pulses at 640 pulses per second) of the contralateral medullary reticular formation (●). Percentage inhibition was measured as the reduction in the number of spikes evoked by a maximal ventral root stimulus of 1 stimulus per second. The squeeze was applied manually for a period of 5 seconds; tetanic stimulation of the reticular formation preceded the volley in the ventral root by 20 msec.

were observed with Renshaw cells; in addition, the sensitivity of these cells to acetylcholine was depressed (Fig. 1). Although recovery after the ejection of noradrenaline was often rapid and at times was followed by enhancement of the sensitivity to excitants, the recovery was occasionally incomplete, with reduction in the amplitude of the spike potential. Depression produced by noradrenaline was not blocked by strychnine injected intravenously (strychnine hydrochloride, 0.05 to 0.1 mg/kg) or electrophoretically (20 to 40 na from a 0.01*M* solution of strychnine hydrochloride in 0.165*M* NaCl). Care was necessary that the currents used to eject strychnine were not of sufficient magnitude to produce extracellular concentrations of this alkaloid which alone reduced the sensitivity of Renshaw cells to excitants.

In marked contrast, the inhibition of Renshaw cells produced either by squeezing the ipsilateral hind paw (*6*) or by tetanic electrical stimulation of the contralateral medullary reticular formation (*7*) was readily blocked by strychnine. Such inhibition was measured by the reduction in the number of spikes evoked by stimulation of the ventral root and was accompanied by a diminution in the sensitivity of Renshaw cells to acetylcholine. Some difficulty was experienced in demonstrating a consistent blocking action of intravenously administered strychnine because of the marked increase in the "background" activity of both the observed neuron and other nearby cells (see also *6*). Inconsistency would also be expected from the polysynaptic nature of the pathways involved in these inhibitions (*6, 7*). However, electrophoretically administered strychnine, which would affect structures only in the immediate vicinity of the tip of the micropipette, readily and reversibly reduced the inhibition of Renshaw cells (Fig. 2). No difficulty was experienced in controlling strychnine efflux from micropipettes used in this study, presumably because of the small size (1 to 2 $\mu$) of the individual barrels of the electrode (*9*).

Thus these postsynaptic inhibitions of Renshaw cells were clearly blocked by strychnine, whereas the depression induced by noradrenaline was not. If the action of strychnine is one of antagonism with inhibitory transmitters at postsynaptic receptor sites, or one of interference with the increase in conductance which is associated with inhibition, these results suggest that noradrenaline is not an inhibitory transmitter acting upon Renshaw cells. As a consequence the noradrenaline "receptors" on these cells, and possibly also upon interneurons, may have no functional significance in synaptic processes. On the other hand, if strychnine blocks these inhibitions by presynaptic action, or if there are other inhibitory pathways converging on Renshaw cells, the effects of which are resistant to strychnine, then noradrenaline may indeed be a spinal inhibitory transmitter.

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**References**

1. A. Fuxe, A. Dahlström, N.-Å. Hillarp, *Int. Cong. Physiol. Sci. 23rd Cong.* (1965), p. 419.
2. A. Lundberg, in *Studies in Physiology Presented to J. C. Eccles*, D. R. Curtis and A. K. McIntyre, Eds. (Springer, Berlin, 1965).
3. D. R. Curtis, J. W. Phillis, J. C. Watkins, *J. Physiol. London* **158**, 296 (1961).
4. K. Krnjević and J. W. Phillis, *Brit. J. Pharmacol.* **20**, 471 (1963); P. Andersen and D. R. Curtis, *Acta Physiol. Scand.* **61**, 100 (1964).
5. I. Engberg and R. W. Ryall, *Life Sci.* **4**, 2223 (1965).
6. V. J. Wilson and W. H. Talbot, *Nature* **200**, 1325 (1963); ——— and M. Kato, *J. Neurophysiol.* **27**, 1063 (1964).
7. R. Granit, J. Haase, L. T. Rutledge, *J. Physiol. London* **154**, 308 (1960); J. Haase and J. P. Van der Meulen, *J. Neurophysiol.* **24**, 510 (1961).
8. D. R. Curtis, in *Physical Techniques in Biological Research*, W. L. Nastuk, Ed. (Academic Press, New York, 1964), vol. 5.
9. D. R. Curtis, *Int. J. Neuropharmacol.* **1**, 239 (1962).

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## Metachromatic Leucodystrophy: Isolation and Chemical Analysis of Metachromatic Granules

Abstract. *The abnormal, cytoplasmic metachromatic granules found in the brain of metachromatic leucodystrophy were isolated in high purity. They show metachromasia by von Hirsch-Peiffer's cresyl violet method. Electron microscopy revealed very slight contaminations by other components, such as mitochondria, myelin, and ribosomes. Chemical analysis of two separately obtained collections has shown that the molar ratio of cholesterol to galactolipids to phosphatides is 1 : 1 : 1. Most of the galactolipids are sulfatides.*

Metachromatic leucodystrophy (MLD) is a genetically determined metabolic disorder primarily involving the nervous system. It is characterized by a large number of abnormal, cytoplasmic granules which show brown-yellow metachromasia when stained by von Hirsch-Peiffer's cresyl violet method (*1*). Abnormally high sulfatide content in white matter of MLD brain was demonstrated by Austin (*2*) and Jatzkewitz (*3*) and subsequently confirmed by many investigators.

The membranous cytoplasmic bodies (MCB) characteristic of Tay-Sachs disease have been isolated in high purity and contain large amounts of gangliosides, the major lipid accumulated in this disease (*4*). The metachromatic granules in brain in MLD differ from Tay-Sachs cytoplasmic bodies in morphology and in their characteristic metachromatic staining property. It would be reasonable to assume that MLD granules might contain large amounts of sulfatides resulting in metachromasia.

The MLD granules were isolated by procedures similar to those devised for isolation of MCB. The procedures were carried out at 4°C. Tissue obtained at autopsy was frozen (1-g samples) and homogenized in 10 ml of 0.05*M* sodium ethylenediamine tetraacetate (EDTA), *p*H 7.5. The homogenate was centrifuged at 1500*g* for 4 minutes, and the

Table 1. Chemical analysis of MLD granules.

| Components | Collection I | | | Collection II | | |
|---|---|---|---|---|---|---|
| | Weight | | Molar ratio | Weight | | Molar ratio |
| | Total (mg) | Dry (%) | | Total (mg) | Dry (%) | |
| Residue | 7.3 | 47.1 | | 4.9 | 46.7 | |
| Total lipids | 8.2 | 52.9 | | 5.6 | 53.3 | |
| Cholesterol | 1.50 | | 1.00 | 1.06 | | 1.00 |
| Galactolipids | 3.88 | | 1.07 | 2.48 | | 0.97 |
| Phosphatides | 2.77 | | 0.93 | 2.03 | | .96 |
| Gangliosides | | | < .06 | | | < .05 |