Histochemical and Functional Correlations in Anterior Horn Neurons of the Cat Spinal Cord

Abstract. *The histochemical reaction for phosphorylase is completely lost from anterior horn neurons rich in phosphorylase within 72 hours after proximal or distal axonal section. Using this new type of axonal reaction as a marking technique in the anterior horn of the seventh lumbar spinal cord segment of the cat, we demonstrated that (i) alpha motor neurons of slow twitch motor units, like those of fast twitch motor units, are rich in phosphorylase and poor in succinate dehydrogenase, and (ii) interneurons and Renshaw neurons are rich in succinate dehydrogenase and poor in phosphorylase. Gamma motor neurons, because of their small size, are considered to be rich in succinate dehydrogenase and poor in phosphorylase. Thus, anterior horn neurons capable of higher firing frequencies (Renshaw neurons, interneurons, and gamma motor neurons) are richer in mitochondrial oxidative enzyme activity as marked by succinate dehydrogenase. Those firing at lower frequencies (both types of alpha motor neurons) are richer in phosphorylase activity and glycogen content and, thus, apparently better equipped for anaerobic glycolysis.*

The anterior horn of the spinal cord in mammals contains alpha and gamma motor neurons, Renshaw neurons, and interneurons (*1*). Alpha motor neurons have been divided into "slow" (type S) and "fast" (type F) on the basis of the distinctive muscle twitch properties of their motor units (*2, 3*). The spectrums of electrical properties of the two types of alpha motor neurons themselves overlap and are not so distinctive (*3*).

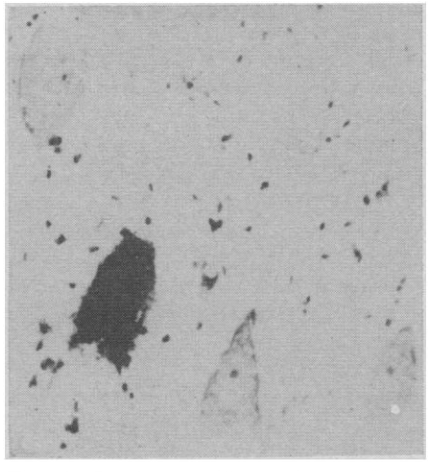

Fig. 1. In the specific territory of the anterior horn in which are located the alpha motor neurons to the soleus and gastrocnemius muscles, one large neuron retains normal phosphorylase activity (deep red on the slide, black in this figure) among three which have lost all such activity (pale blue gallocyanin counterstain on the slide). The tissue was tested for phosphorylase activity 3 days after the nerve to the soleus was spared while all other motor branches of the peroneal and tibial nerves were cut. The preserved neuron presumably innervates slow twitch muscle fibers of the soleus.

Anatomic and physiologic studies have shown that the different neurons are topographically intermingled, and that the larger are mainly alpha motor neurons whereas the smaller are a mixture of gamma motor neurons, Renshaw neurons, and interneurons (*1, 4–7*). The alpha motor neurons of slow motor units are considered to have somas somewhat smaller than those of fast motor units, based on axonal diameter, axonal conduction velocity, ventral root spike, and input resistance of the soma (*3, 8, 9*).

We have shown that the histochemical reactions for phosphorylase and succinate dehydrogenase (SDH) in anterior horn neurons of the cat are related to neuronal size (*10*). Neurons larger than approximately 30 μm are rich in phosphorylase and poor in SDH, whereas neurons smaller than 30 μm are rich in SDH and poor in phosphorylase. The glycogen content and uridine diphosphate glucose glycogen transferase activity parallel phosphorylase activity. (Sixteen other histochemical reactions failed to distinguish different anterior horn neurons.) It was thought that the fast alpha motor neurons, because they are the largest, are rich in phosphorylase and poor in SDH and that gamma motor neurons, Renshaw neurons, and interneurons, because of their small size, correspond to the other histochemical group. However, the slow alpha motor neurons and perhaps some interneurons could not be characterized histochemically because their sizes are not yet known precisely.

The presence of phosphorylase was determined histochemically in fresh-frozen 20-μm serial sections of the cat seventh lumbar (L-7) spinal cord segment removed under Nembutal anesthesia (*10*). Seven animals were examined 1, 2, 3, or 4 days after section of one L-7 root; two others were examined 10 to 20 days after section of one sciatic nerve. In another group of four animals, the spinal cord was studied 3 days after all the muscles innervated by the tibial and peroneal nerves, except the soleus, were denervated in one leg and the nerve to the soleus muscle alone was cut in the other leg.

By 72 hours after section of either the root, the sciatic nerve, or muscular branches of nerves, the phosphorylase reaction of the corresponding neurons was equally and completely lost from their perikarya. This new type of axonal reaction occurred earlier and more constantly than the classical chromatolysis of Nissl substance (rough endoplasmic reticulum), but of course it could only be observed in those neurons normally rich in phosphorylase.

After complete section of the L-7 root, no phosphorylase stain remained in any anterior horn neurons of the corresponding segment. After section of the sciatic nerve, the neurons corresponding to this nerve (*5*) were devoid of phosphorylase. Selective denervation of the soleus muscle, which in the cat contains only slow twitch motor units (*3, 11*), resulted in loss of phosphorylase from scattered neurons larger than 30 μm located in the specific territory of the anterior horn described by Romanes (*12*) as that of alpha motor neurons to the soleus and gastrocnemius muscles of the cat. Conversely, sparing the nerve to the soleus and cutting all other motor branches of the peroneal and tibial nerves resulted in persistence in the same specific territory of some phosphorylase-rich neurons larger than 30 μm among

Table 1. Correlations of histochemical and functional properties in cat anterior horn neurons.

Neuron	Presumed soma diameter	Phosphorylase-rich and SDH-poor (> 30-μm soma)	SDH-rich and phosphorylase-poor (< 30-μm soma)
α-Motor, fast twitch motor units	Large	+	−
α-Motor, slow twitch motor units	Medium–large	+	− (?)
γ-Motor	Small	− (?)	+
Interneurons and Renshaw neurons	Small	−	+

the majority of that size which had lost phosphorylase (Fig. 1).

We conclude that: (i) Fast alpha motor neurons, the largest in the motor neuron pool, belong to the group that is rich in phosphorylase and poor in SDH. (ii) Slow alpha motor neurons, considered on the basis of indirect evidence to be somewhat smaller than the fast ones (*3, 8, 9*), are also mainly if not entirely of this histochemical type, although we cannot exclude that a few might be of the opposite type. (iii) Renshaw neurons and other interneurons, whose axons do not form part of the motor roots, are not included in the phosphorylase-rich group because no neurons in the anterior horn remained positive for phosphorylase after root section; apparently they are small neurons rich in SDH and poor in phosphorylase. (iv) The majority if not all of the gamma motor neurons, because they are thought to be less than 30 μm in diameter (*4–9*), appear to be rich in SDH and poor in phosphorylase (Table 1).

The similarity between alpha motor neurons innervating fast twitch muscle and those innervating slow twitch muscle in regard to phosphorylase and SDH histochemical reactions is in sharp contrast to the histochemical differences (*13*) of the muscle fibers of those muscles, wherein the fibers of fast twitch muscle are mainly rich in phosphorylase (and glycogen) and poor in SDH, whereas those of slow twitch muscle are rich in SDH and poor in phosphorylase (*14*). The functional properties of the two types of alpha motor neurons are not as clearly distinct from each other as are the functional properties of the muscle fibers they innervate (*2, 3*). However, the differences between functional properties of alpha motor neurons as a group compared with those of Renshaw neurons, other interneurons, and gamma motor neurons are prominent and parallel the histochemical properties. Renshaw neurons (*7, 15*), other interneurons (*7*), and gamma motor neurons (*4, 7, 8, 16*) are capable of higher firing frequencies (also perhaps fire relatively more often). They are richer in mitochondrial oxidative enzyme activity as marked by SDH activity. In being better equipped for mitochondrial oxidative enzyme activity they resemble type I (slow twitch, red) muscle fibers. Alpha motor neurons fire at lower frequencies (*3, 4, 7, 8, 15, 16*) (also perhaps less often) and are richer in phosphorylase activity and glycogen content. Thus all the alpha motor neurons are like type II (fast twitch, white) muscle fibers in being better equipped for anaerobic glycolysis.

JUSTINIANO F. CAMPA
W. KING ENGEL
Medical Neurology Branch, National Institute of Neurological Diseases and Stroke, Bethesda, Maryland 20014

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Expression of Allelic Immunoglobulin in Homozygous Rabbits Injected with RNA Extract

Abstract. *Ribonucleic acid extracts obtained from lymph nodes of immunized rabbits homozygous for the* b^4 *or* b^5 *allele of light chain immunoglobulin allotypes were injected intravenously into nonimmunized rabbits homozygous for the alternate allele. Approximately 30 percent of the plaque-forming cells in the spleen yielded plaques with immunoglobulin M antibody possessing the allotype of the RNA donor. The allotype of the RNA donor was also found in the IgG immunoglobulin of lymphoid cell lysates as well as in the IgG isolated from the serum. These results suggest that the injected RNA has an informational role in the in vivo synthesis of immunoglobulins by host lymphoid cells.*

An informational role of immunologically active RNA extracts was suggested by the experiments of Adler, Fishman, and Dray (*1*) in which it was shown that the IgM (*2*) antibody to T2 phage synthesized by RNA-treated homozygous lymphoid cells in culture possessed light chains with allelic allotype genetic markers (*3*) characteristic of the RNA donor. Using sheep red blood cells (SRBC) as the antigen and the localized hemolysis in gel technique (*4*) to assess IgM and IgG antibody formation (*4*) by single cells, we extended these observations and reported (*5, 6*) that RNA extracts of lymphoid cells from immunized homozygous rabbits, with the b^4 or b^5 light chain allele (*2*), convert spleen cells from nonimmunized homozygous rabbits, with the b^5 or b^4 allele, respectively, to produce antibody of foreign light chain allotype. When the RNA was extracted from immunized rabbits 5 days after a single intravenous injection of 4×10^8 SRBC, "direct" plaque-forming cells (PFC) were observed; these could be inhibited by 2-mercaptoethanol (2-ME) or goat antibody to IgM (goat anti-IgM) but not by goat antibody to the Fc fragment of IgG (goat anti–Fc-IgG) indicating that the antibody produced by the PFC were of the IgM class (*5*). When the RNA was extracted from immunized rabbits 18 to 24 days after the first of several intravenous injections of SRBC, "indirect" PFC were observed by developing the plaques with goat anti–Fc-IgG; the "indirect" PFC were not inhibited by 2-ME or by goat anti-IgM but were inhibited by excess goat anti–Fc-IgG, an indication that the antibodies in the plaques were of the IgG class (*6*). The RNA was extracted from lymphoid cells homozygous for one allele, b^4 or b^5, controlling light chain allotypes of immunoglobulin, whereas the spleen cells were homozygous for the other allele, b^5 or b^4 (*5, 6*). By use of antibodies to the b4 and b5 allotypes, the allotypes of the IgM or IgG antibody produced in the plaques were identified (*5, 6*). Practically all of the "direct" plaques and most of the "indirect" plaques of the converted spleen cells possessed IgM or IgG antibody, respectively, with the allotype characteristic of the donor of the "immune" RNA extract (*5, 6*). Moreover, the allotype of the RNA donor