Variations in the cellular incorporation of [3H]proline

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Injections of [3H]proline into various CNS nuclei in the cat and into the rat dorsal column nuclei labeled terminal targets of those regions despite the fact that only glia (and not neuronal somata) were labeled at the injection site. In contrast, although injections into cat CNS fiber tracts labeled glial cells surrounding the axons, neither the axoplasm, somata or terminals of those axons were labeled. In addition, injections into cat PNS dorsal root ganglia labeled ganglion cells. These results support previous findings suggesting that [3H]-proline is incorporated into a class of molecules in the CNS (but not PNS) that is transferred from glia into neuronal somata (but not axoplasm).

When tritiated proline ([3H]Pro) is injected into the dorsal column nuclei (DCN) of adult cats, it labels macroglial cells but fails to label neurons within DCN11. Despite the lack of labeling of the soma of DCN neurons, such injections label both their axoplasm2 and their terminal target regions13.

Although this labeling pattern in cat DCN occurs under a variety of conditions (including very short survival times)3, the pattern has been reported only briefly for only a few regions in the cat brain; that is, in the lateral reticular nucleus9, cerebellar cortex6 and inferior olive7. One purpose of the present study, therefore, was to use light microscopic autoradiographic techniques to determine the generality of this labeling pattern by examining whether similar patterns occurred after [3H]Pro injections of DCN in other species (adult rat) or of other nuclear regions in cat central nervous system.

One of the conclusions supported by the results cited above is that the injected [3H]Pro is incorporated...
Fig. 2. Light microscopic autoradiograms illustrating labeling patterns produced in cat somatic sensory cerebral cortex (A), ventrobasal complex of the thalamus (B), lateral cervical nucleus in the spinal cord (C) and L4 dorsal root ganglion (D) 1 h (A) or 24 h (B, C, D) after injection of $[^3]$H]proline. Thin arrows in D indicate a few examples of unlabeled satellite cells. Thicker arrow points to labeled axoplasm in the axon of a ganglion cell. Bar = 50μm.
into a class of glial molecules that is first transferred from the glial cells into adjacent neurons before being further transferred, by axonal transport, to the terminal targets of those neurons. If true, then \(^{3}H\)Pro labeling patterns might vary depending upon the nature of neuron-glial cell associations in the region being injected. This possibility was tested by examining the light microscopic autoradiographic labeling patterns after \(^{3}H\)Pro injections into various central nervous system fiber tracts and into peripheral nervous system dorsal root ganglia in cats.

Injections of \(^{3}H\)Pro into CNS nuclei, CNS fiber tracts and dorsal root ganglia were made in 7 adult cats, 2.5-4.5 kg, of either sex, anesthetized initially with sodium thiopental (i.v.) and maintained with halothane gas. Survival time was 24 h except where noted. In some cats, several sites along the neuroaxis were injected with \(^{3}H\)Pro; in others, only one site was injected. Injected nuclei were the lateral cervical nucleus, parts of the medullary reticular formation, the inferior olive (1.5 h survival), reticular nucleus (thalamus), lateral hypothalamus, ventral posterolateral nucleus (thalamus), somatosensory cerebral cortex (1 h survival) and cerebellar cortex. Injected fiber tracts were the dorsal columns (spinal cord), pyramidal tract (in medulla), 7th cranial nerve (in pons), internal arcuate bundle and internal capsule. Injected dorsal root ganglia (some with 48 h survival) were the L4-S1 ganglia.

The labeled proline was either \(L-[2,3-{^3}H(N)]\) (spec. act. 11.1 Ci/mmol) or \(L-[2,3,4,5-{^3}H(N)]\) (spec. act. 139.1 Ci/mmol, New England Nuclear). It was concentrated to 20 µCi/µl by drying under flowing N\(_2\) and reconstituted in saline. Total amounts injected into each site at 0.02 µl/min through glass micropipettes ranged from 2 to 7 µCi in volumes from 0.1 to 0.35 µl.

Anesthetized cats were perfused transcardially with 10% formalin. The brain and spinal cord were removed and stored in 10% formalin with 30% su-

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Fig. 3. Light microscopic autoradiograms illustrating labeling patterns in cat fiber tracts 24 h after injection of \(^{3}H\)proline. A is from the dorsal columns of the spinal cord; B is from internal arcuate fibers in the medulla. Bar = 50 µm.

Fig. 4. Light microscopic autoradiograms illustrating labeling patterns produced in rat dorsal column nuclei 24 h after injection of \(^{3}H\)leucine (A) or \(^{3}H\)proline (B). Bar = 50 µm.
crossed for at least 24 h before freezing and cutting into 25 μm-thick sections (coronal plane). Some dorsal root ganglia were frozen and cut as above; others were embedded in paraffin and cut at 10μm. Sections were processed for light microscopic autoradiography as described in an earlier study1 and stained with thionin.

In 3, chloropent-anesthetized, adult rats, the DCN was injected with 0.2 μl of either [3H]Pro or [3H]Leu (L-[3,4,5]-3H(N)]-spec. act. 140 Ci/mmol). After 24 h, the rats were anesthetized with sodium pentobarbital followed by perfusion and tissue preparation as described above for cats.

The labeling patterns in rat DCN (Fig. 4) and in all of the injected cat central nervous system nuclear regions (Fig. 2 A-C) were virtually identical to that in cat DCN (Fig. 1). Thus, only small cells (i.e. presumably glial cells) were labeled at the injection sites, and terminal regions of neurons in those sites were moderately labeled. Although the labeling pattern in fiber tracts was similar, in that only presumed glial cells were labeled at the injection site (Fig. 3), injections of the tracts did not result in labeling of their neuronal somata or terminals. In contrast, the labeling pattern in dorsal root ganglia (Fig. 2D) differed significantly from that in the central nervous system. Here ganglion cell somata and axoplasm were densely labeled and satellite cells were sparsely labeled, in a pattern similar to that for amino acids other than proline in the central nervous system (e.g. Fig. 1A).

These results support the conclusion that preferential incorporation of exogenous proline by glial cells is a general characteristic of the adult mammalian central, but not peripheral nervous system. These incorporation differences might reflect the separate embryological origins of CNS and PNS cells12. The differences might also be related to differences in the structural relationship between neurons and non-neuronal cells in the CNS and PNS5. For example, CNS neurons might not be able to incorporate [3H]Pro because some sort of a glial cell barrier, perhaps in the form of a net of glial processes around the soma8,10, impedes the uptake of [3H]Pro by the soma. Such ‘nets’ do not appear to exist in the PNS, except perhaps in the enteric nervous system5.

The results also support the conclusion that although [3H]Pro-labeled glial molecules appear to be transferred from glial cells into adjacent neuronal somata2, they are not transferred into adjacent neuronal axoplasm. This lack of transfer could reflect either an inability of glial cells in fiber tracts to transfer molecules across myelin or, perhaps, an inability of elements in the axoplasm to add transferred [3H]Pro-labeled molecules into the axonal transport system (see discussion on p. 60-61 in ref. 2).

In addition to its possible functional significance with regard to the transfer of molecules between glial cells and neurons, the [3H]Pro incorporation pattern in the CNS is important for neuroanatomical tracing studies. The present study provides additional evidence that [3H]Pro can be detected in the terminal regions of neurons in a variety of CNS nuclei, despite the lack of labeling of those neurons at the injection site. Thus, [3H]Pro remains useful as an axonal ‘tracer’. However, the results from studies using [3H]Pro should be interpreted with some caution, since the mechanism by which the labeling of terminal fields occurs may be indirect.

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