CONSIDERATIONS ON NEUROTROPHIC RELATIONS IN THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM

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Abstract. The evidence for the existence of neurotrophic (non-impulse) influences is reviewed especially with respect to choice of adequate parameters and of differentiation of neurotrophic influences from other mechanisms affecting intercellular relations. The importance of growth processes in neurotrophic control is stressed, the mechanisms being apparently similar in the central and peripheral nervous system. Collateral sprouting as a compensatory response of the neuron on one hand and the old age changes at neuromuscular synapses resulting in “silent” synapses and later “silent” terminals present phenomena indicating mechanisms of growth and arrest of growth processes in neurotrophic control.

Nature of neurotrophic relationships

Increasing evidence has been accumulating in support of the existence of two basic communication systems between neurons and between neuron and muscle cell. Extremely rapid events with durations of milliseconds that occur with the transmission of nerve-impulse activity, synaptic facilitatory and inhibitory reactions, could, relatively early, be differentiated from long-term “neurotrophic” events, which “maintain and restore structure and functional capacity of the cells” (for ref. see 13, 17, 19, 33).

Both communication systems are evidently closely interconnected and therefore an exact differentiation of mechanisms related to impulse transmission and transmitter release from those related to neurotrophic
"maintenance" mechanisms has become increasingly difficult. These difficulties have been due to the fact that the parameters of trophic functions were often not exactly defined and that intercellular regulations between neurons and between neuron and muscle cell also implies many non-neural regulations such as hormonal mechanisms or cell interactions in the immune response (see 4). Study of these relationships and their possible interaction with neural mechanisms started only recently. With the exact knowledge of the neuro-transmitter involved, as well as the almost exclusively focal contact at the synapse, the nerve-muscle cell relationship has been the preferred system for studies of trophic relationships.

However, there is much evidence suggesting that the basic mechanisms are probably the same in the CNS. The notion, that "maintenance" and/or dependence of the muscle on nerve cell cannot be explained solely by neuronal activity related to transmitter release has been strengthened by observations on (i) synthesis, transport, and release of both proteins and neurotransmitters (40, 53, 61); (ii) general mechanisms of neurosecretion, i.e., secretion of diverse active proteins in neurons (59); (iii) axoplasmic transport of proteins moving with widely varying rates (45, 54, 70); (iv) the complex nature of the synaptic apparatus involving macromolecular systems in synthesis of vesicles and surface recognition (see 61) and release of transmitter by exocystosis (see 9) and suggesting mechanisms of information transfer between cells beyond alterations in excitatory states produced by fast transmitter release, (v) effects of the distal nerve stump on muscle after nerve section independent of nerve impulse transmission. Onset of degeneration of presynaptic terminals, muscle membrane changes, development of hypersensitivity to ACh, failure of transmitter release as well as many intracellular denervation changes depend on the length of the axon (for rev. 19, 20, 33), whereas no such dependence could be observed with respect to onset of contraction properties of denervated muscle (28) (see Fig. 1). These observations stress the significance of axoplasmic transport for neurotrophic maintenance, not directly connected with nerve impulse activity, and suggest a different mechanism for regulation of contractile properties of muscle, dependent primarily on nerve impulse activity. (vi) Apparently non-specific growth promoting influence of nerves in amphibian limb regeneration, independent of impulse conduction (see 60). (vii) "Trophic" effects of neurons on muscle in tissue and organ culture without synaptic transmission and even without contact between nerve and muscle cell (33, 42). (viii) Differentiation of intrafusal muscle fibres after efferent motor denervation resp. abolition of motor nerve-impulse activity (71). (ix) Morphological, phy-
Fig. 1. Number of intact end plates (first bar), levels of glycogen after injection of glucose (second bar), of activity of proteolytic enzymes (third bar), cholinesterase-activity (fourth bar) and full contraction time (fifth bar) in muscles innervated by the peroneal nerve 24 hr after section of the nerve high up in thigh, expressed in % of corresponding levels in the same muscle denervated close to nerve entry (= 100%). The peroneal nerve was sectioned high up in the thigh on one, and close to the muscle on the other side. Note later onset of denervation changes in muscle with long nerve stump, but lack of such relation with respect to contractile behavior (20, 28).

Multiple regulation of muscle cell function and neuronal specificity

There is a surprisingly high adaptation capacity of skeletal muscle cell with respect to structural, contraction and biochemical properties (for rev. see 8, 21). The classical cross-union of nerve experiments (6) demonstrated a transformation of muscle with respect to contractile...
behavior from slow into fast and vice versa. The neuronally induced change was first thought to be due to some trophic factor released from the nerve (6). However contraction properties and the histochemical muscle fibre pattern can also be changed in a denervated muscle by stimulation in vivo (44, 49) and in vitro (26). Speed of muscle contraction appears to be therefore primarily regulated by impulse activity, the ensuing repeated depolarizations of the muscle membrane, and not primarily to neurotrophic influences as suggested before (see Fig. 1). The pattern (frequency) of nerve impulses appears to be of special importance (44, 58). The spread of ACh sensitivity after denervation may also not be primarily related to the absence of a “neurotrophic” factor (51) since it was shown that denervation supersensitivity reverts to the normal state after stimulation of a denervated muscle (10, 43). Whatever, the implications, changes in speed of contraction or ACh sensitivity cannot be considered adequate parameters for testing neurotrophic influences. There is increasing evidence for a multiple regulation of properties of muscle, including neural (i.e., impulse and non-impulse), myogenic, hormonal, vascular, immunological and peripheral influences (21). A good opportunity for the study of myogenic (properties encoded in the muscle cell itself) and hormonal influences on muscle is afforded by androgen-sensitive (“target”) muscles. It was possible to differentiate the hormonal and neural influences in cross-union experiments of nerves to the androgen-sensitive levator ani (LA) muscle. Hetero-innervation of the LA muscle reversed the muscle fibre pattern accordingly to the foreign nerve supply even in an atrophic muscle after castration; testosterone administration alone did increase fiber size but did not change the fiber pattern (30). If a slow muscle is transplanted into the place of the androgen-sensitive (fast) LA muscle of the rat, contraction time of the slow muscle is transformed to a fast one in response to the “fast” nerve supply of the pudendal nerve, now reinnervating the slow muscle. However, the grafted skeletal muscle does not acquire sensitivity to androgens, i.e., no change of contraction time or weight is observed after castration or testosterone administration, as is the case in the androgen-sensitive (“target”) muscle (32). The hormone sensitivity of this “target” muscle is thus primarily of myogenic origin. However, hormones may also have an effect by some kind of feed-back system involving the motor neuron.

For instance testosterone, which is known to primarily affect receptors in the muscle cell, rapidly increases the synthesis of ChAc in the hormone sensitive levator ani muscle of castrated rats (39).

The main and best known transformations of muscle cell are due to the specific properties of the neuron, inducing the wide change of
properties mediated by the new or "foreign" nerve supply. A sensory nerve fiber innervating a muscle will produce plexus-like formations as in the skin and will not enter the motor end-plate (18). Furthermore, a nerve fibre cross-innervating slow-tonic or fast-phasic muscles of the chicken will produce their specific type of multiple or focal innervation pattern according to source of nerve supply (34). How far this considerable presynaptic specificity of the neuron is able to induce related functional (e.g., change of contraction properties) and structural changes will depend on other factors, especially the time at which the change of innervation took place. We do not yet know much about the importance of "myogenic" factors. In any case, the substantial neuronal specificity of spinal motor neurons is remarkable and is also expressed by homogeneity of motor units in which properties of muscle fibers, belonging to one unit are closely matched to the properties of the neuron, innervating them (12).

There is a relative uniformity of peripheral motor neurons compared with the great multiplicity of central neuron types. A very high specificity of central neurons is generally assumed on the basis of the formation of specific nerve connexions in the developing and regenerating nervous system (63). The mechanisms underlying selective neuronal interconnections, in which apparently some kind of chemospecificity operates, are still not yet clear. Only lately alterations of neuronal specificities have been reported (16), suggesting possibilities of changes in intercellular relationships during development and repair of the CNS (14). It remains to be seen to what extent neuronal specificity is mediated by impulse and/or non impulse activities. Interaction with other, e.g., myogenic influences can, however, not be ignored.

**Growth processes and neurotrophic control**

The close coupling of impulse and (neurotrophic) non impulse activities of the neuron and the lack of direct biochemical identification of neurotrophic factors have resulted in some scepticism concerning the existence of neurotrophic functions. This scepticism has been raised especially with respect to findings that impulse activity as such may play a decisive role in regulation of chemosensitivity of the muscle membrane and of morphogenesis of the postsynaptic membrane (10, 35, 37, 43), the latter defined often as the result of an inductive action of the nerve (see 33, 38). However, we may again draw attention to observations on the non-synaptic role of neurons in promoting limb regeneration in amphibia (41) in regulation of ChE (42) and in "induction" of the postsynaptic membrane (see 38). Growth phenomena are involved in these experiments and it seems appropriate therefore to point out
some of these events in the central and peripheral nervous system. Two processes will be considered: (i) "spontaneous" degeneration and regeneration, (ii) collateral regeneration. Rapid and early perinatal degeneration and involution of neurons and muscle cells does take place during normal development.

Neuronal cell death in neurogenesis is a wide-spread event and is most probably due to the fact that more cells are produced than finally needed (55) and that only those gaining successful contact are maintained. The changes apply to both neuro-muscular and neuronal contacts. "Spontaneous" axon-degeneration (5) was found also during later stages of peripheral nerve maturation (57) and a concurrent process of regression and regeneration of synapses has been observed also in the adult mammalian CNS (62).

The scope of the possible events is very wide indeed. For instance in the androgen-sensitive levator ani muscle of the female rat complete perinatal degeneration (a "programmed cell death") is observed and rapid involution of the pudendal nerve follows (31). Both processes can be stopped by perinatal injection of testosterone. On the other hand in the process of rapid and complete degeneration of the flight muscles of some insects terminal axons remain intact until old age (29). On the other hand boutons ending on injured neurons disappear from the cell surface after axotomy but restore contact again when the axon reinnervated the muscle (67). One may postulate a continuous linked degeneration and regeneration process related to remodelling of axon terminals and some of these data suggested a synaptic plasticity as a structural basis for a growth theory of learning (13). The laws and the significance of such processes are not yet clear. It can, however be pointed out, that these observations are consistent with concepts of a perpetual growth and proximo-distal transport of axonal agents (69), mechanisms basically related to neurotrophic functions. A continuous process of end-plate regression and regeneration has been reported (3). With aging, muscle fibres are lost before degenerative changes in the axons can be observed. This leads apparently to collateral regeneration (22) and/or degeneration of endplates with no replacement (65). The ultrastructural changes in senescent endplates concern different stages. Figure 2 shows the endplate of a senescent muscle in which spontaneous transmitter release had been reduced to a small fraction of the original one (27). We suggest the term "relative silent synapse". Figure 3 shows the very rare event in which destruction of the postsynaptic apparatus is observed. The terminal axon is still intact but withdrawn from the destroyed muscle membrane. The term "silent terminal" is suggested (24). It will be interesting to see whether analogous mechanisms will be observed also in central synapses.
Fig. 2. Electron micrograph of the levator ani muscle of a three-year-old male rat, showing motor endplate with two axonal terminals. Note high occurrence of synaptic vesicles (sv) and mitochondria (M). Junctional infoldings (ji) of longitudinally cut muscle fiber (m). “Relative silent synapse” \( \times 45.000 \). (55).

Fig. 3. Electron micrograph of the soleus muscles of a 34-month-old rat. The small axonal terminals are detached from the transversally cut muscle fiber (m), the junctional infoldings (ji) are destroyed with tendency of disappearing. The cytoplasmic processes of the Schwann cell (S) covers the axonal terminal. “Silent terminal”. \( \times 30.600 \). (55).
Fig. 4.  
A: Cross section of the extensor digitorum longus muscle of rat 10 weeks after denervation, stained for phosphorylase activity. Note small diameter of muscle fibres with low and uniform enzyme activity. (Magn. 200 ×)

B: Cross section of the same muscle freely grafted after 2 weeks of denervation and fixed to chest wall below the cutaneus max. muscle, examined 4 weeks after grafting and stained for phosphorylase activity. Note increase of diameter of muscle fibres with "type grouping" and high enzyme activity in peripheral area of denervated muscle reinnervated by collateral sprouts. (Magn. 200 ×.) (67).
With such regression the capacity for collateral regeneration may have been lost.

Collateral sprouting can be defined as a compensatory response of a neuron, aiming to establish new contacts. It is apparently a general event, both in the central and peripheral nervous system. This phenomenon was first described in the skin (68). After section of a cutaneous sensory nerve, the neighbouring intact axons sprout and invade the denervated area. In young animals the functional significance may be considerable, as recovery of sensation in the denervated area at least to a great part can take place as a result of this "extension of axons" into a denervated area. Collateral sprouting has been described in detail in muscle (11) and also in sympathetic ganglia, autonomic effector tissues and within the CNS (46, 52, 56, 66).

The phenomenon is best studied in muscle with focal neuromuscular contact. Normally an innervated muscle will not accept new innervation; however, after denervation, an end plate may be formed at any place in the denervated muscle fibre (25) and there seems to be a close association between sensitivity to ACh and ability to accept new contact (50). However, tissue culture work has given no evidence that ACh release from the nerve or presence of functional ACh receptors on a muscle surface, is a necessary precondition for nerve muscle synapse formation (33). Additional innervation can be achieved after implantation of a foreign and temporary interruption of the original nerve, thus producing muscle fibres with two endplates (23). Such hyperinnervation could also be achieved after a chronic reversible nerve block (36). The notion that in such compensatory collateral sprouting trophic factors, conveyed by proximo-distal axoplasmic flow, are involved is strengthened by experiments in which, after blockage of impulse conduction in nerve, sprouting and enlargement of peripheral motor and sensory fields in salamanders could be observed (1). The fate and capacity of such "collateral" neurons varies considerably. Figure 4 shows that after free grafting of the extensor digitorum longus muscle over the chest wall and below skin-muscle a mixed pattern of muscle fibres showing larger diameters is established. Correspondingly speed of contraction in the grafted muscle is considerably increased (E. Gutmann and A. Montgommery, in preparation). This transformation of the denervated grafted muscle is apparently due to collateral regeneration, the source of which we were not able to ascertain. Implantation of a fast nerve into a slow muscle leads, however to marked transformation of muscle fibre pattern (7). The experiments on additional innervation in the ocular muscle of fish (48) suggested that terminals from the original nerve can render foreign innervation ineffective thus producing "synaptic repression" in foreign junct-
ions previously functioning. In mammalian muscle, however, the foreign synapses were found to remain functional (15).

The evidence for the existence of sprouting in the CNS is convincing (46, 52, 56, 66). Observations on regeneration in marginal zones of a "denervated" thalamic region were attributed to a continued axonal growth into the denervated zone (66). The "reoccupation of a synaptic territory" vacated by lost contacts in the CNS may thus follow analogous trends of collateral regeneration as in a peripheral synapse and neurotrophic (non-impulse) activities may play a decisive role. Moreover, heterotypic synaptic regeneration has been shown to be functionally effective (47, 64). Observations on peripheral and central "synaptic regeneration" may therefore be useful in indicating how the long-term neurotrophic communication systems might operate.

It must, however, be remembered, that these growth processes are only one aspect of neurotrophic relations and that the considerable variations in capacity and efficiency of these processes makes their study complicated.

REFERENCES


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