ANALYSIS OF THE TRANSMISSION OF EXCITATION FROM NERVE TO MUSCLE IN CURARIZED DOGS AT DIFFERENT AGE PERIODS

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Investigations in the author's laboratory have shown that, judging from physiological finding, the myoneural junctions in skeletal muscles mature gradually during ontogenesis and acquire the typical characteristics of the adult orgaism only at the time when locomotor acts are learned and stabilized, [1-10]. This gradual nature of the maturation of the myoneural junctions during postnatal ontogenesis has also been revealed by morphological investigation [11, 13-15].

Because of the importance of this problem, an attempt was made to continue the analysis of the mechanisms of maturation of the myoneural junction during ontogenesis. If the myoneural junctions mature gradually, this obviously must influence the blocking action of curare at different age periods.

EXPERIMENTAL METHOD

Experiments were carried out on 46 dogs and puppies ranging from 1 day old to adult animals. The puppies aged up to 1.0-1.5 months were anesthetized with ether and the adult dogs and the puppies over 1.0-1.5 month old with morphine and ether. The test object consisted of the sciatic nerve and gastrocnemius muscle. Buried silver electrodes, 5 mm apart, were applied to a segment of the nerve isolated from the center and were connected to a "Multistim" electronic rectangular pulse generator (Disa), giving impulses with a duration of 0.5 msec. Direct stimulation was carried out at various distances from the point of entry of the nerve into the muscle. The stimulation needle electrodes, coated with varnish (except at the very end), from 1.5 to 3 mm apart depending on the age of the dog, were either buried in the muscle or placed in contact with its surface. As during indirect stimulation, so also during direct the presence or absence of a block was tested only to single and double stimuli. The action potentials from the muscle in response to direct and indirect stimulation were detected by means of bipolar needle electrodes. The distance between the detecting electrodes and the stimulating electrodes in each experiment varied from 5-6 to 30 mm depending on the size of the muscle, and because of the necessity of ensuring the presence of a reaction to direct stimulation. The comparative closeness of the electrodes (stimulating and detecting) – made it impossible to exclude the possibility of an artifact of stimulation, but this did not interfere with the recording of the muscle action potentials. The action of competitive curare (pachycurare), an infusion of alkaloids of depolarizing curare (leptocurare), and lysthenon (succinylcholine, ditilin), was investigated. Curare (infusion) was injected in a dose of between 0.2 and 1.0 ml/kg and lysthenon in a dose of between 2 and 6 mg/kg. All that was attempted in the investigation was to assess the presence or absence of blocking effect depending on the age of the animal, and no attempt was made to investigate the thresholds of the blocking doses.

EXPERIMENTAL RESULTS

From the results obtained the experimental animals could be divided into three age groups. Group 1 included puppies aged up to 16-18 days (29 animals), group 2 – puppies aged from 20 days to 2.0-2.5 months (10 animals), and group 3 – puppies over 3 months old and adult dogs (7 animals).

In the animals of age group 3, 2-3 min after intravenous injection of both pachycurare and leptocurare, action potentials from the muscle ceased to be recorded in response to indirect stimulation. In response to direct stimulation of the muscle action potentials continued to be recorded but their amplitude was low-

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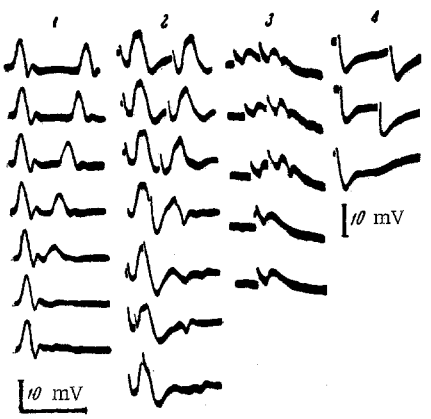

Fig. 1. Action potentials of a muscle in response to indirect and direct stimulation in puppies of age group 1 before and after curarization. 1 and 3) Indirect excitability before and after curarization; 2 and 4) direct excitability before and after curarization (artifacts can be seen in 4).

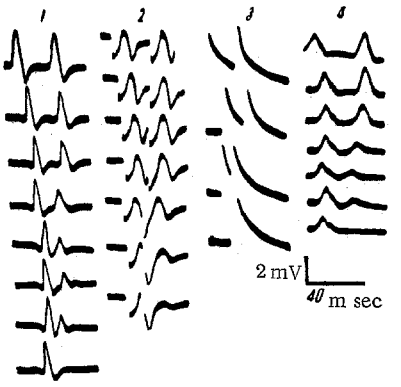

Fig. 2. Action potentials of a muscle in response to indirect and direct stimulation in puppies of age group 2 before and after curarization. 1 and 3) Indirect excitability before and after curarization; 2 and 4) direct excitability before and after curarization (artifacts can be seen in 3).

ered by 50%. Hence in the animals of age group 3, indirect excitability (excitability of the subsynaptic membrane) disappeared on curarization but direct excitability (excitability of the postsynaptic membrane) of the muscle was preserved.

In the puppies of age group 1 after intravenous injection of curare (competitive and lysthenon) a complete block to indirect stimulation did not develop. After curarization, in response to indirect stimulation the muscle remained capable not merely of generating action potentials, but also of contacting. However, the amplitude of the action potentials was reduced by 50-60%, and in individual cases, by 70%; the intensity of the contractile reaction was also diminished. Because of the decrease in the amplitude of the action potential in response to indirect stimulation, it cannot be concluded that blocking is absent at an early age. It would be more correct to speak merely of partial blocking. The muscle reacted to direct stimulation either by a very weak local stational contration, not becoming speading in character, or it did not react at all. Hence in the puppies of age group 1, in contrast to adult animals, after curarization indirect excitability persists while direct disappears (Fig. 1). After the 18th-20th day (in the puppies of age group 2) the reaction of the muscle to indirect stimulation disappeared completely after curarization and the muscle gradually developed the ability to respond to the action of direct stimulation by the generation of an action potential and a speading contration (Fig. 2).

A. G. Ginetsinskii and N. M. Shamarina [12] studied the dynamics of the spatial change in sensitivity of muscle tissue to acetylcholine in fetuses and animals during the first day of life. They found that in fetuses and newborn rabbits the skeletal muscles possess tonic properties and marked sensitivity to acetylcholine throughout their extent. With each successive day the part of the muscle fiber reacting to acetylcholine becomes smaller, and on the 10th day it corresponds to the zone of the synapse.

Diamond and Miledi [17] confirmed the findings of Ginetsinskii and Shamarine in investigations on rats [12]. They found that in rats in the early stages of development chemoreception of acetylcholine is generalized over the whole surface of the muscle fiber. During ontogenesis the chemoreceptive zone of the muscle tissue gradually contracts and by the 15th day of life it is confined to the region of neuromuscular synapse.

It may be postulated that the loss of direct excitability of the muscle tissue in the present experiment (excitability of the postsynaptic membrane away from the future subsynaptic region) in puppies under 16-18 days of age was due to the fact that at this period curare (both competitive and depolarizing) blocks the whole chemoreceptive surface of the muscle fiber. It may be assumed that at an early age, in response to direct stimulation, the muscle is also excited because of stimulation of the nerve ending within it. From

this point of view direct stimulation at an early age is evidently also indirect. It was natural to try to explain why after the action of blocking substances the generalized chemo-receptive apparatus of the muscle fibers ceases to react at an early age to electrical stimulation. Meanwhile, the results indicating that indirect excitability is preserved after curarization suggests that at an early age the subsynaptic region has not yet developed to maturity and the chemoreceptive properties of this region in the early stages differ from those properties of the rest of the postsynaptic region of the muscle fiber. The cause and the physiological significance of these differences must receive further analysis. If we remember not only the facts obtained from the study of the results of curarization, but also the laboratory investigations cited above, it must be accepted that final maturation of this region takes place in dogs at the age of 2.0-2,5 months.

The results obtained provide an explanation of clinical observations which have been reported. Stead [18], for instance, states that a much larger dose of depolarizing relaxants per kilogram body weight is required to produce apnea in newborn infants than in adults. Churchill-Davidson [16] describes the high resistance of newborn infants to decamethonium (leptocurare). He found only a partial block, which could be removed by anticholinesterase preparations of neostigmine type. I. A. Arshavskii [8] reports that in dog fetuses, joined to the mother by the umbilical cord, the subcutaneous injection of curare causes disappearance of reflex reactions stimulation of various receptive fields of the skin surface. The loss of reflex reactions in this case may be due to partial blocking of impulses not only in the myoneural junctions, but also in the synapses of the central nervous system.

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