The effect of tetrodotoxin on the synaptic and extrasynaptic membrane in frog skeletal muscle

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Summary. Tetrodotoxin resistant sodium channels have been shown to operate in frog muscle membrane, which are responsible for local action potentials. These channels are located mainly in synaptic regions, and their distribution on the membrane is controlled by neurotrophic factors.

It is known that the rate of rise of the action potential (AP) in the synaptic area of skeletal muscle fibers in the frog and in the rat is greater than the rate in extrasynaptic areas[1,2]. Voltage-dependent sodium channels in the neuro-muscular junction in rats are known to be more resistant to tetrodotoxin (TTX) than those on the extrasynaptic electrogenic membrane of muscle fibers[2]. After denervation, AP resistant to TTX are first recorded in the synaptic region and only later on the extrasynaptic membrane[2,3]. This suggests that the membranes of the muscle fibers in the end-plate region and in the extrasynaptic sites differ in their functional organization, and that these differences are under nervous control. In our investigation this problem was studied using frog muscle.

Experiments were performed on sartorius muscles of the frog, *Rana temporaria*, at room temperature during autumn and winter. We used the conventional intracellular microelectrode technique. Localization of the microelectrode within the synaptic region was inferred from the occurrence of miniature end-plate potentials (MEPP) with a rise-time of less than 1,3 msec. Denervation was performed by removal of a 3–4-mm nerve branch segment supplying the muscle, approximately 2 cm from it. In other experiments, instead of denervation, a solution of 30 mM colchicine (CCh) (Merk) was applied for 30 min to the same nerve site. Ringer solution contained (in mM): NaCl 115, KCl 2.5, CaCl$_2$ 1.8 and phosphate buffer pH 7.2.

Application of TTX (Sankyo) at a concentration of 5×10^{-7} M suppressed the AP development in muscle fibers, thus confirming the previous data for frog muscles[4]. However, in 9 cases out of 30 (30%) when recording in the extrasynaptic region and in 34 cases out of 37 (92%) when recording in the synaptic region, depolarizing pulses produced responses which may be classified as a graded local response (fig. 1). The reasons are as follows: unlike the AP, the amplitude of local potentials is graded with depolariza-

tion; hyperpolarizing pulses do not produce a similar downward response; after replacement of Ringer by sodium-free TRIS solution, these responses did not appear; TEA (5×10^{-2} M) did not suppress the responses suggesting that the potential transients were not evoked by delayed potassium conductance; in calcium-free solution (containing 4 mM MgCl$_2$) local responses were recorded in 12 out of 14 fibers studied; destruction of the tubular system, by bathing the muscle in Ringer with 400 mM glycerol (1 h) followed by a wash in normal Ringer solution[5,6] had no significant effect of the development of the local response. Polarization of muscle fibers up to a membrane potential of +20 mV produced no reversal of the local response. The equilibrium potential for the acetylcholine response in phasic fibers of the frog is known to be about -15 mV[7]. Hence the development of graded local responses could not be attributed to the action of acetylcholine released by nerve endings under electrical stimulation. The transformation of AP into the local responses occurred within about 5 min of the addition of TTX. An increase in the period of TTX application up to 1.5 h or an increase of TTX concentration up to 1×10^{-5} M influenced neither the magnitude nor the frequency of occurrence of the local response. This suggests that the phenomenon described is not due to channels which are not readily reached by TTX because of the length of the diffusion pathway.

It would seem that the recorded response is a local potential associated with the passage of current through voltage-dependent sodium channels in the surface membrane. The mechanism of this response is similar to that of AP generation, yet operates in the presence of TTX.

The experiments have shown that the local response is greatest in the synaptic area and decreases progressively with distance from the end-plate. The area within which the local response could be recorded varied widely; it averaged 1 mm along the fiber, but in some instances it was as much as 2 mm (fig. 2). In 30% of the fibers investigated a small local response could be observed everywhere on the muscle membrane.

The sartorius muscle of the frog is not homogeneous; the fiber composition on the external surface of the muscle differs from that on its inner surface[8]. It is possible that local response development and the area within which they can be recorded depend upon muscle fiber type. However, little
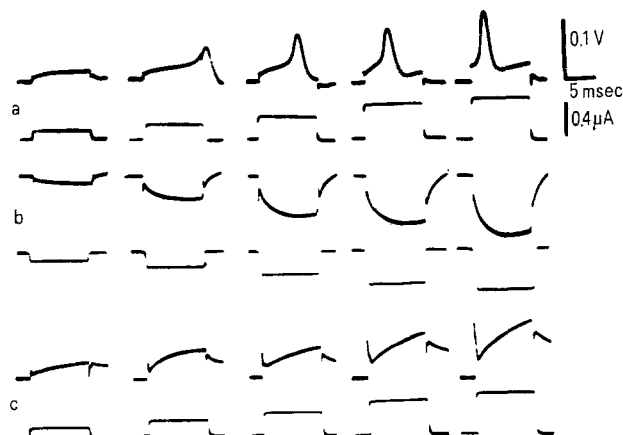

Figure 1. Microelectrode recordings of responses to depolarizing (a) and hyperpolarizing (b) current pulses of different amplitude in the synaptic region and to depolarizing pulses in the extrasynaptic (c) region of muscle fiber. TTX 5×10^{-7} M.
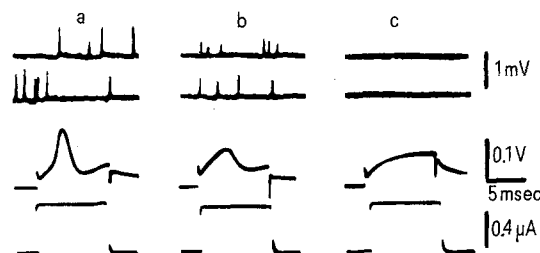

Figure 2. Miniature endplate potentials (above) and responses to depolarizing current pulses recorded at 750 mkm (b) and 1500 mkm (c) from the synaptic region (a), determined by maximum amplitude of miniature potentials. TTX 5×10^{-7} M.

difference in responses of fibers from the 2 sides of the muscle was observed.

13–15 days after denervation, the number of muscle fibers generating local responses in the extrasynaptic area in the presence of TTX $(5 \times 10^{-6}$ M) increased from 9 in 30 to 13 in 25 cases (i.e. from 30 to 52%). After the same period from CCh application, the number of responding fibers rose to 17 out of 33 (i.e. also to 52%).

It is known that sodium channels located in the synaptic region of rat muscle are more resistant to TTX than those outside the synaptic area, but all are blocked by raising the toxin concentration[2]. In our experiments an increase in TTX concentration to 10^{-5} M did not eliminate the local responses.

The rate of rise of the AP within the synaptic region is higher $(225 \pm 7.8$ V/sec (mean $\pm$ SE, 21 fibers)) than that observed extrasynaptically $(197 \pm 6.4$ V/sec, 63 fibers). The difference is significant according to the t-test $(p < 0.02)$. This result supports other data for amphibian[1] and mammalian muscles[2]. It may be assumed that in the synaptic region of frog skeletal muscle there are 2 types of voltage-dependent sodium channels; one is nonresistant, and the other resistant to TTX. It is possible that the extrasynaptic muscle membrane has a uniform density of TTX nonresistant channels, whereas in the synaptic region there are additional channels resistant to TTX. This might contribute to the increased density of sodium channels on the postsynaptic membrane thereby affording a more rapid redistribution of charge.

In frog muscle fibers denervation does not cause development of TTX resistant AP[4]. But the present data show that muscle denervation, by nerve cutting or by blocking axoplasmic transport with CCh, leads to an increase in the number of muscle fibers capable of generating local responses on the extrasynaptic membrane in the presence of TTX. This suggests that the localization of TTX resistant sodium channels in the synaptic area is determined by nervous control, as is well known in the case of cholinoreceptors. To eliminate this control it is sufficient to block axoplasmic transport by colchicine, rather than to inhibit the transmission of excitation.

Frog muscle fibers studied in our experiments differ from muscle fibers in mammals in 2 aspects. 1. An increase in TTX concentration does not completely block the development of local AP; 2. In 30% of tested muscle fibers, TTX resistant local responses occur on the extrasynaptic membrane even without denervation.

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Face representation linked with literacy level in colonial American tombstone engravings and Third World preliterates' drawings. Toward a cultural-evolutional neurology

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Summary. Among colonial North-American artisans, subgroups of South-Americans, Indonesians and New Guineans, a close correspondence exists between illiteracy rates and specifically spatially inaccurate representations of the upper face configuration, a characteristic also seen in the pre-literate period of 'neolithic' art, in early individual development, and in certain pathological regressions. Common to the configuration both of lexical signs and of the face is a specific spatial-relational ratio and orientation. Accurate representation of both configurations appears to be neuro-developmentally linked, within a cultural context, and consistent with a novel position that the 'ontogeny' of such cognitive functions recapitulates their prevailingly culturally determined 'phylogeny'.

This study raises the question of the non-identity between the mental and the graphic representation of a specific object; the human face. The findings here reported will show that pre-literates, i.e. persons untrained in the accurate graphic representation of lexical signs, also showed a significant percentage of inaccurate graphic representations of the human face exclusively. These preliterates were not known to have had problems with the perception and recognition (mental representation) of the face. Other objects were graphically represented without essential distortions. (The characteristic exception of finger misrepresentation only by certain remote cultural groups is of special interest – see later.) Findings from studies of face recognition by Carey et al.[2,3] and by Yin[4] also point to the face configuration's quite specific neurological mediation, the existence of which is supported by the present results. In addition, these point to an association between the neurodevelopmental phases of the graphic representation of the face and of lexical signs within a cultural context. Such a clustering of functions appears to be analogous to that found in the early development of mankind and of the individual, as well as in certain states of pathological regression.

Historical data can be drawn from the pre-literate period of 'neolithic art'[5] that was produced up to about 4 millenia ago. Its most characteristic sign, found world-wide, is a specific and consistent spatial-relational distortion: an obliteration of the narrow or indented area of the bridge of the nose, in short, a depiction of a 'neolithic face' (fig., NGr and NGl). This specific sign persists in historically younger populations within subgroups that have had neither training for literacy nor, it seems, for depiction of the human face[6-8].

On the basis of this clue from historical observation, performance was rated on a task (drawing or gravestone engraving) requiring the pictorial representation of the uniquely oriented human face configuration, containing the complex