RE-EXAMINATION OF MUSCLE FIBRE TYPES IN A MAMMALIAN DIAPHRAGM

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The ventral and dorsal regions of the rat diaphragm, when processed histochemically for cholinesterase, revealed, on the basis of the type of nerve-endings, two types of muscle fibres. Majority of them were innervated through large, round, darkly stained ‘en plaque’ type of nerve-endings and should therefore be considered to be of the twitch type. A few others were seen to be multiply innervated and each nerve-ending was of the ‘en graspé’ variety. Such fibres should therefore be considered as the tonic type. The ventral and dorsal regions of the rat diaphragm, therefore, are mixed tonus-twitch in nature.

It is now well known that the mammalian diaphragm is composed morphologically of three different regions viz. dorsal (vertebral), lateral (costal) and ventral (sternal) regions and that each region has a characteristic cellular organization. Of interest is the fact that the biochemical composition of both ventral and dorsal regions shows striking similarities with that of the purely tonic muscles such as avian Mm. latissimus dorsi anterior and brachialis. Both, ventral and dorsal regions of the diaphragm* and tonic muscles* have a high lipid but low glycogen content and exhibit a low activity of succinate dehydrogenase. Although these regions have not been studied separately, electrophoretic pattern of lactate dehydrogenase isoenzymes of the whole diaphragm matches with that of a mixed tonus-twitch muscle such as avian M. deltoideus minor*.

With reference to the innervation of the muscle fibres it is now a commonly accepted fact that the fast contracting twitch fibres bear, generally, a single round or ellipsoid plate like ‘en plaque’ nerve-ending of a large diametered nerve fibre. In contrast to this, the slow contracting tonic fibres are multiply innervated. Several
small-diameter nerve fibres terminate on every tonic muscle fibre, with the result each tonic fibre bears a series of nerve-endings along its length. Such nerve-endings are grape-like ‘en grappe’ nerve-endings. Despite this, and the facts mentioned earlier, it must be said that the studies made so far, on cellular organization, view the diaphragm to be composed of red, white and intermediate fibres, all the three being innervated exclusively through ‘en plaque’ motor end-plates. In view of these discrepant facts it was decided to undertake the present study to ascertain whether the ventral and dorsal regions of the diaphragm had in fact a mixed population of twitch as well as tonic fibres.

MATERIAL AND METHODS

20μm thick longitudinal fresh frozen cryotome sections of ventral and dorsal regions of diaphragm of freshly killed adult albino rats (Rattus norvegicus albigans, Haffkine strain) were taken and processed for histochemical demonstration of cholinesterase employing the method of Karnovsky and Roots using acetyl thiocholine iodide as substrate. The pH of the incubation medium was 5.2. The temperature at which incubation was carried out was 37°C and the incubation time varied from 1 to 6 hours.

RESULTS AND DISCUSSION

A processed longitudinal section of ventral region of diaphragm, observed under low magnification, revealed the presence of round motor end-plates placed on the outer margins of the central portions of most of the fibres. Across the central part of each section was to be found a zig zag row of these end-plates (Fig. 1). Only a detailed observation revealed the row to be interrupted at places where one could see in an over-incubated section, distinct ‘en grappe’ type of nerve-endings, associated with only those muscle fibres which were devoid of heavily stained round ‘en plaque’ motor end-plates (Fig. 2). Moreover these fibres were multiply innervated. The presence of ‘en grappe’ endings is not immediately apparent in a preparation incubated for a short time for the reason that the overall acetylcholinesterase activity is weaker at the ‘en grappe’ endings as compared to the ‘en plaque’ variety. A clear grape-like structure is seen to advantage under high power (Fig. 2).

Similarly, the longitudinal section of the dorsal region of the diaphragm, when incubated for a short duration, revealed only ‘en plaque’ nerve endings. The same
sections when incubated for a prolonged time brought out the faintly stained 'en grappe' nerve-endings which could then be seen readily under high magnification (Fig. 3). It must be noted here that a cursory observation using low magnification is likely to draw one's attention towards heavily stained 'en plaque' endings and against this heavy back-ground the 'en grappe' variety goes unnoticed, giving an impression of there being only 'en plaque' nerve-endings present. A clear grape-like structure is seen to advantage under high power (Fig. 3).

![Image](image.png)

Fig. 1. Photomicrograph of longitudinal section of ventral region of rat diaphragm processed for cholinesterase. Note the zig-zag row of darkly stained 'en plaque' type of nerve-endings. The 'en grappe' endings are lightly stained and are scattered along the length of remaining muscle fibres. X 37

Fig. 2. L.S. of the ventral region of the diaphragm processed for cholinesterase. Note the presence of two types of fibres. The majority bear intensely stained large oblong motor end-plates (p). Others show multiple innervation and each nerve-ending is in the form of a bunch of grapes i.e. comprising of a few discrete dots (g). X 120
The present findings, therefore, confirm in part the earlier report of Naik who described the presence of three types of fibres viz. red, white and intermediate in the ventral and dorsal regions of the diaphragm. All the three being innervated through 'en plaque' motor end-plates. However, the present work suggests that in addition to the three types of twitch fibres described by him, the presence of a fourth type viz. tonic type must also be taken into account.

Fig. 3. L.S. of the dorsal part of the diaphragm processed for cholinesterase showing intensely stained round 'en plaque' nerve-ending marked (p) and weakly stained 'en grappe' nerve-endings marked (g). X 120 readily accessible lateral portion of the diaphragm which was being studied by this author.

It must be mentioned here that Gunther did report the presence of tonic fibres in rat diaphragm. Hess, however, could not find a single multiply innervated fibre in rat diaphragm. Possibly, it was studies underway to see whether ventral and dorsal regions of the diaphragm from other mammals also have a mixed population of tonus as well as twitch fibres. For the present, it may be said that future pharmacological and physiological studies on diaphragm should also take the presence of tonic fibres in the ventral and dorsal regions of the diaphragm into consideration and should, moreover, clearly specify the region being studied. The present work also points out the need of studying mammalian musculature in greater detail to dispel the commonly held view of mammalian musculature to be scarce in tonic fibres. To date, only four muscles, viz. extraocular, stapedial, tensor tympani and rat cremaster have been reported to possess tonic fibres. The need for studying the mammalian musculature in greater detail is called for.

REFERENCES