

are the most useful experimentally (KIDD and KUČERA¹⁰) because these usually supply only 1–3 spindles. Tandem spindles do occur in these muscles. PORAYKO and SMITH⁴ found no tandem spindles in rat lumbricals. Axon diameters in supplying branches varied from 2–12 μ in normal and de-efferented preparations.

Sensory innervation. The central placing and form of the primary receptor ending do not differ from those in cat. Secondary receptor endings are found in a zone 100 μ long on either side of the primary. As in cat those secondaries next to the primary may take the form of rings and spirals, but most simply branch to end in fine filaments. Branches from some secondary endings appear to end in the region occupied by the primary. Only 10% of spindles have a single primary, while 10% have one secondary, 60% two secondaries and 20% three secondaries.

Motor innervation. Three forms of fusimotor ending are present in this muscle.

(1) A plate ending confined to the polar regions is structurally similar to extrafusul motor end plates, though less than half their size. There is a nucleated sole plate and a discrete subneural apparatus is revealed by cholinesterase staining. The axons supplying these plates have a diameter less than half that of axons supplying extrafusul muscles, but occasionally axons of similar diameter may supply extrafusul end plates. Axon diameter at the level of the spindle may bear no relation to that in the nerve trunk.

(2) Another form of plate ending occurs in the juxtaequatorial region, and is twice the size of the polar plates, has no nucleated sole plate and is supplied by an axon twice the diameter of axons supplying polar plates. The ending takes the form of several short tapering branches and knobs.

(3) Typically this is a multiterminal ending, occurring in the juxtaequatorial region. However, the ending is pleomorphic and may range from a single filament to many ramifying branches of different diameter. The whole juxtaequatorial region stains diffusely for cholinesterase activity, like those first found by CÖERS^{11,12} in rat rectus abdominis spindles. Some more intensely stained areas

are associated with the terminals of the 2nd and 3rd endings described above.

Discussion. These motor endings presumably correspond to the p_1 , p_2 and trail endings described in cat by BARKER¹³. The conformation of endings in rat spindles is however less elaborate than those in cat. PORAYKO and SMITH⁴ found only 2 forms of fusimotor endings in rat lumbricals. STEG¹⁴ working on other caudal muscles more distal than the intertransverse found spindles innervated by single γ -efferents.

BARKER^{13,15} states that p_1 plates are supplied by mixed (β) axons. Mixed innervation of the first plate endings described above has not yet convincingly been demonstrated in this muscle. This does not preclude the possibility as the techniques used will not demonstrate axon branching in the nerve trunk. These plate endings are however often supplied separately from the rest of the spindle by nerve branches which otherwise supply solely skeletomotor end plates.

Zusammenfassung. In den Muskelspindeln der Quermuskulatur im Rattenschwanz kam eine einzige primäre sensorische Endung in 10% der Spindeln vor, die übrigen hatten 1–3 sekundäre Endungen, 2 platte Endungen und 1 Endung unbestimmter Art.

MARGARET H. GLADDEN¹⁶

*The Physiology Laboratory,
The University of Liverpool (England),
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¹⁰ G. L. KIDD and J. KUČERA, *Experientia*, in press (1969).

¹¹ C. CÖERS, in *Symposium on Muscle Receptors* (Hong Kong University Press, Hong Kong 1962).

¹² C. CÖERS and J. DURAND, *Archs. Biol., Liège* 67, 685 (1956).

¹³ D. BARKER, in *Myotactic, Kinesthetic and Vestibular Mechanisms* (J. and A. Churchill Ltd., London 1967), p. 3.

¹⁴ G. STEG, *Acta physiol. scand.* 61, Suppl. 225 (1964).

¹⁵ M. N. ADAL and D. BARKER, *J. Physiol.* 177, 288 (1965).

¹⁶ Medical Research Council Junior fellow.

On the Possible Existence of Muscarinic Cholinoreceptors on the Postsynaptic Membrane of the Frog Muscle

It is known that cholinoreceptors of the postsynaptic membrane of skeletal muscle of vertebrates have all the pharmacological characteristics of nicotinic receptors. However, these muscles are markedly affected by some muscarinomimetics, and the effect of ACh may be blocked by muscarinolytics, for example by atropine (AS)¹. The question arises whether there are differences in the specificity of cholinoreceptors of the skeletal muscle or whether all cholinergic substances act on identical cholinoreceptors of the postsynaptic membrane.

The ability of AS to shift the point of reversal (E_r) of the endplate potential towards the Na equilibrium potential^{2,3} and to change its shape¹ could be explained if it were possible to establish that receptors with muscarinic properties were present among the receptors of the postsynaptic membrane. It could be supposed that one

type of receptor is connected with Na and the other with the K permeability of the postsynaptic membrane. AS blockage of the 'K cholinoreceptors' would then change the relationship $\Delta gNa/\Delta gK$ and cause a shift in E_r . It could be assumed that the shape of the normal endplate potential results from the effect of ACh on both types of receptor. In the presence of AS, only the effect on the nicotinic receptors would remain and the shape of the endplate potential would be altered.

In order to confirm this assumption, the dose-response curves for butyrylcholine (BCh), a nicotinomimetic drug,

¹ R. BERÁNEK and F. VYSKOČIL, *J. Physiol.* 195, 493 (1968).

² T. V. POTAPOVA, *Biofizika* (Russ.), in press (1968).

³ L. G. MAGAZANIK, F. VYSKOČIL, *Experientia*, in press.

and methylfurmetide (MF), a muscarinomimetic drug, were compared. The drugs were applied iontophoretically from double-barrel micropipettes at the endplate region of the *m. sartorius*. The cholinergic region of the endplate is much more sensitive to BCh than to MF, which accords well with earlier findings⁴. However, the course of the dose-response curves runs in parallel. The administration of tubocurarine ($6 \times 10^{-7} M$) into the muscle bath decreased the sensitivity of the endplate to both BCh and MF approximately four times. The same results were obtained with AS ($6 \times 10^{-5} M$) (Figure 1). The dose-response curves of BCh and MF were shifted in parallel. These results provide evidence against the hypothesis that there are receptors on the endplate which have muscarinic properties, because in this case the antagonism of tubocurarine would have to be selective towards BCh, and that of AS towards MF. It is apparent that these cholinergic substances act on an identical type of cholinergic receptor of the frog muscle as in the case of invertebrates^{4,5}.

A study of the effect of AS on the shape of the BCh potentials was made in the course of the experiments. Whereas the MF potential only decreases in amplitude, AS not only causes the same decrease in amplitude of the BCh potential, but also markedly prolongs it (Figure 2; see the vertical lines drawn through the peak and half peak horizontal lines). In order to analyse this fact, a study was made of the effect of AS on ACh, propionylcholine (PCh) and BCh potentials. It was found that the prolonged duration appears only in the case of the BCh potential. This effect is not changed by the addition of $2 \times 10^{-6} g/ml$ neostigmine sulphate into the medium and has no relation to the E_r of ACh and BCh potentials. E_r

values were identical ($-14.2 \pm 1.8 mV$ in 7 experiments with double-barrel micropipette) and AS did not affect the E_r of both substances ($-15.0 \pm 2 mV$ in 7 experiments; for method see³). As our results show that the character of the postsynaptic action of BCh is analogous to ACh, we may conclude that the difference in the shape of the potentials and the effect of AS on the shape of the BCh potential can be explained by differences in the conditions of the molecule fixation of these substances on the postsynaptic membrane and in their diffusion in the synaptic region.

Thus it would be possible to understand the differences in the behaviour of analogues which have so similar a chemical structure – choline esters of acetic (ACh), propionic (PCh), and butyric (BCh) acids. Perhaps AS hinders the diffusion and access to receptors of molecules with a longer hydrophobic chain (BCh) and this slows down the initiation and decreases the BCh-potentials. However, direct experimental evidence is necessary to support this assumption⁶.

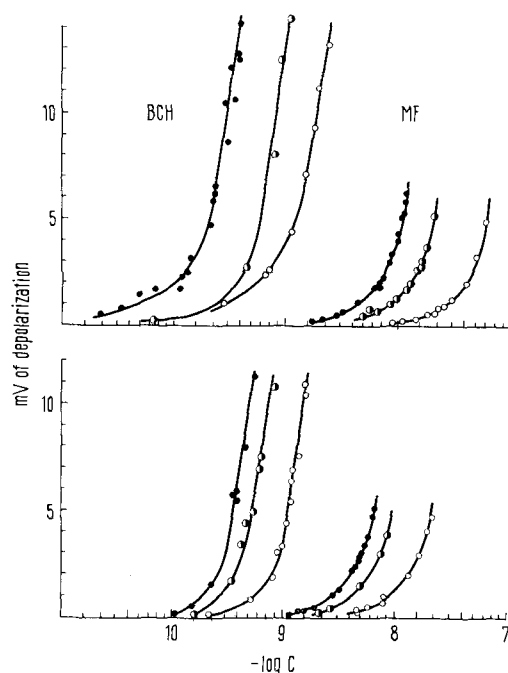


Fig. 1. Dose-response curves of iontophoretically applied butyrylcholine (BCh) and methylfurmetide (MF) before the application of $6 \times 10^{-5} M$ atropine (upper part) and $6 \times 10^{-7} M$ tubocurarine in the bath (lower part ●—●); shift of curves after 15–20 min exposition to drugs (○—○); recovery after 40 min (◐—◐). Potential changes were registered by intracellular glass microelectrodes filled with 2.5 M KCl. The ordinate shows the charge transferred by micropipette (coulombs – C).

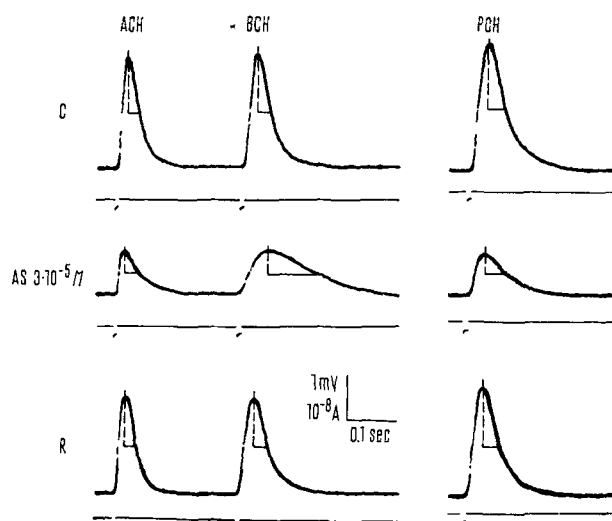


Fig. 2. The effect of atropine sulphate (AS $3 \times 10^{-5} M$) on the amplitude and shape of acetylcholine (ACh), butyrylcholine (BCh) and propionylcholine (PCh) potentials evoked by the iontophoretic application of these compounds on the endplate zone. C, control; R, recovery (40 min). Double-barrel electrodes were used. Temperature 20°C. (Retouched.)

Zusammenfassung. An den postsynaptischen Muskelzellmembranen werden Befunde erhoben, welche darauf hinweisen, dass qualitativ keine Differenzierung zwischen muskarin- und nikotinartigen Rezeptoren möglich ist.

L. G. MAGAZANIK and F. VYSKOČIL

Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences, Leningrad (USSR), and Laboratory of Cellular and Comparative Neurophysiology, Institute of Physiology, Czechoslovakian Academy of Sciences, Praha-Krč (Czechoslovakia), 20 December 1968.

⁴ L. G. MAGAZANIK, *J. Evolut. biochem. physiol.* 1, 220 (1965).

⁵ L. G. MAGAZANIK, *Physiology and Biochemistry of Invertebrata* (Ed. E. M. KREPS, Leningrad 1968), p. 205.

⁶ This work was performed between February and July 1968.