Effects of cholinergic and adrenergic agents and their antagonists at the neuromuscular junction of the cat extraocular muscles

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A report of early and recent investigations of the effects of cholinomimetics, neuromuscular blocking agents, and sympathomimetics on cat extraocular muscles.

Duke-Elder and Duke-Elder first reported on the action of choline and nicotine on the mammalian extraocular muscle; this was followed by a study by Brown and Harvey on the cat extrinsic eye muscle. Since then very little investigation has been reported on mammalian extraocular muscle, except by Breinin. Recently, more detailed investigation of cat extraocular muscle has been reported following administration of cholinomimetics, neuromuscular blocking agents, and certain sympathomimetics. It is the purpose of this publication to present a summarized account of these and earlier investigations.

Cholinomimetics

Observations of Duke-Elder and Duke-Elder have been confirmed following nicotine and choline. Following intra-arterial administration, both agents cause a sustained contraction of the cat extraocular muscle. Succinylcholine (SCh), acetylcholine (ACh) and a potent cholinomimetic, 1,1-dimethyl-4-phenyl-piperazinium (DM-PP), also cause a sustained contractile response (Fig. 1). However, they vary in their effectiveness. SCh is the most active of these agents; nicotine is the least effective. Parasympathomimetic agents, muscarine and acetyl-β-methylcholine, were also able to evoke a weak contractile response of extraocular muscle (Fig. 2).

Neuromuscular blocking agents

Among the agents which interrupt neuromuscular transmission at the motor end-plate region by causing a sustained depolarization, succinylcholine appears to be the most effective agent. Succinylcholine, when administered in small doses, either intravenously or intra-arterially, evokes a sustained contraction of extraocular muscle of the cat; at the same time it may cause a small depression of neuromuscular conduction in tibialis anterior or gastrocnemius muscle (Fig. 3). Only following larger doses was SCh found to cause a depression of the twitch response of extraocular muscle. The effect of decamethonium on the extraocular muscle is essentially similar to that caused by suc-
Fig. 1. Effects of cholinomimetics on superior oblique muscle of the cat (weight, 2.2 kilograms) in vivo. Upper trace in each pair of records is the time of injection and the lower trace is the maximal twitches elicited indirectly once every 2 seconds. At SCh, 0.5 μg of succinylcholine was administered intra-arterially, at ACh, 25 μg of acetylcholine, at DMPP, 5 μg of DMPP and at N, 50 μg of nicotine.

Fig. 2. Effects of muscarine and epinephrine on superior oblique muscle of the cat (weight, 2.7 kilograms) in vivo. Upper record is arterial blood pressure, middle record is the time of injection, and the lower record is the maximal twitches of superior oblique muscle elicited indirectly once every 2 seconds. At MUS, 5 μg of muscarine and at EPI, 1 μg of epinephrine injected intra-arterially.

cinylcholine, the only difference being a longer duration of the effect of decamethonium compared to that of SCh (see Fig. 3). This may be due to a fairly rapid hydrolysis of SCh by nonspecific esterase in plasma, whereas decamethonium is not metabolized to any significant extent.

The sustained contractile response evoked by cholinomimetics and by depolarizing blocking agents has been referred to as contracture. Gasser defined contracture as a contractile response which is reversible and prolonged but associated with no propagated conduction in the muscle fiber. In our study, following small doses of cholinomimetics, the sustained contractile response was accompanied by repetitive motor unit potentials throughout the duration of the response. Fig. 4 and the lower record in Fig. 5 show such a response following succinylcholine injection. Brown and Harvey had reported similar observations following the administration of acetylcholine. Thus the contractile response produced by small doses of cholinomimetics...
Fig. 3. Effects of succinylcholine and decamethonium on superior oblique and gastrocnemius muscles of the cat (weight, 2.4 kilograms) in vivo. In each pair, the upper record is maximal twitches of gastrocnemius and the lower record is maximal twitches of superior oblique muscle elicited by indirect supramaximal single shock nerve stimulation once every 2 seconds. At SCH, 10 μg per kilogram of succinylcholine and at Cw, 10 μg per kilogram of decamethonium injected intravenously.

Fig. 4. Effects of 0.5 μg of succinylcholine administered intra-arterially on the tension and the motor unit potentials of superior oblique muscle of the cat (weight, 2.5 kilograms). Abscissa is time in seconds following the injection and the ordinate is the tension developed during a sustained contractile response. Insets at each point represent motor unit potentials of the muscle at respective time. Calibration, 50 μv.
Fig. 5. Same experiment as in Fig. 4. Lower record represents the effect of 1 μg and the upper record, 10 μg of succinylcholine administered intra-arterially. Calibration, 50 μv.

cannot be solely a contracture. However, the contractile response of extraocular muscle following large doses may be due, at least in part, to a contracture. The repetitive activity of the motor unit was evident only during the rising phase of the contractile response (Fig. 5). Repetitive action potentials, following SCt, may have originated, as in other twitch muscles, from the presynaptic activation at the motor nerve terminals with antidromic invasion of the entire twitch motor units, and/or from the diffusely multiply-innervated fibers. But Hess and Pillar reported that diffusely multiply-innervated muscle fibers were solely contracture fibers in that the added ACh caused only depolarization and shortening proportional to the magnitude of depolarization. On the other hand, Bach-y-Rita and Ito have recently presented evidence that these multiply-innervated fibers exhibit both local contracture and a propagated action potential if the depolarization is sufficiently great. This would indicate that diffusely multiply-innervated fibers are analogous in function to certain contracture fibers of the birds. However, with our extracellular recording technique we could not distinguish between repetitive asynchronous propagated activation of individual muscle fibers and a true contracture. The resolution of this aspect awaits further studies with intracellular microelectrodes. There do appear to be differences in mechanism of muscle activation with different doses of SCt.

Regarding the sensitivity of these muscles to neuromuscular blockade produced by depolarizing agents, much larger doses of these agents are required compared to the dose necessary for the blockade in the limb muscles. We had also found that following small doses of cholinomimetics there was a potentiation of indirectly elicited twitches of the extraocular muscle. This potentiation may have been due to repetitive activation of twitch motor units in response to a single nerve volley. Following large doses of SCt, the depression of maximal twitches may have been the consequence of any or all of a number of events: e.g., SCt does produce neuromuscular block in twitch fibers, but the depression could also have been the result of an inability of the muscle fibers to produce further tension when some fibers were already shortened by SCt. A third possibility for the depressed twitch tension may have been the result of a possible presynaptic action of SCt on the motor nerve terminals. SCt will depolarize the membrane of the nerve terminals, thus depressing the transmitter release which will result in the reduction of twitch height.

Thus it may be stated that twitch fibers of extraocular muscle appear to be different from those of the leg muscle, e.g,
soleus, in their ability to respond to SCh with sustained repetitive activity. This may be due to relative insensitivity of extraocular muscle to neuromuscular block produced by SCh, or there may be less "adaptation" of motor nerve terminals to SCh. With respect to the sustained activation of muscle fibers by the cholinomimetics, the extraocular muscles appear to behave similarly to the intrafusal muscle fibers of mammalian muscle spindle as assessed on the basis of spindle afferent discharge. It is worth noting that no typical muscle spindle has been observed in cat extraocular muscle.\textsuperscript{19}

It is known that mammalian eye muscles are more sensitive to curare than other skeletal muscles. However, we found that, following curare, the indirectly elicited twitch response of the extraocular muscle was less depressed than similarly evoked response of the limb muscle (Fig. 6). Similar observations have been reported by Katz and Eakins.\textsuperscript{8} Further, following curare, the response of the extraocular muscle to tetanic nerve stimulation was

![Graph showing effects of tubocurarine on superior oblique and gastrocnemius muscles of the cat (weight, 2.0 kilograms) in vivo. In each pair, the upper record is arterial blood pressure, the middle record is maximal twitches of superior oblique muscle elicited indirectly once every 2 seconds, and the lower record is maximal twitches of gastrocnemius muscle elicited indirectly once every 2 seconds. At TC, 600 μg of tubocurarine was injected intravenously. At SCh, 1 μg and 5 μg of succinylcholine was injected intra-arterially, and at T, tetanus, 30 per second for 5 seconds was applied.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933621/)
altered so that a previously smooth tetanic response was converted to a nonfused type (Fig. 6). This may have certain significance. In man, version such as convergence may be mediated over slow diffusely innervated muscle fibers as suggested by Alpern and Wolter. If curare in small doses depressed these fibers, diplopia could occur even though some ability to move the eyes is retained. This difference in fiber sensitivity could also be the basis of conversion, by curare, of smooth, fused tetanic response to a nonfused response due to depression of the slower fibers with lower fusion frequency, leaving fast twitch fibers less affected. A further difference in sensitivity of extraocular muscles to curare was noted when the effect of curare was tested on twitches and tetani. Unlike leg muscle, where tetanic response is more markedly affected by curare than the twitch response, the extraocular twitch and tetanic responses were almost equally affected by curare, as determined by the percentage of reduction in the control response.

The difference in sensitivity of the extraocular muscle of the cat as compared to human eye muscle may be due to species difference and not to different frequency of discharge in the motor nerves to extraocular and limb muscles.

Gallamine (Flexidil), unlike curare, affects extraocular muscle to the same degree as limb muscle. Sympathomimetic amines

Many investigators have observed the stimulatory effect of sympathomimetic amines on extraocular muscle. However, there is no consensus as to the effects and functional role of these agents. In our study, epinephrine was found to cause a small contractile response of extraocular muscle of the cat (Fig. 2). The contractile response induced by epinephrine could not be abolished by prior administration of atropine. Histologically no data are available to show the presence of smooth muscle elements in extraocular muscle, except the blood vessels. As regards innervation, Wolter suggested the presence of sympathetic fibers in human extraocular muscles. A question may be asked as to what effect the contraction of other smooth muscle elements in the orbit, induced either by electrical stimulation of sympathetic nervous system or exogenous sympathomimetic amines, will have on the tension of extraocular muscle. The observations of Brecher and Mitchell indicated that stimulation of cervical sympathetic nerve evoked a contraction of nictitating membrane of the cat without any change in the tension of extraocular muscle. From our observations, where the superior oblique muscle was completely isolated from other structures in the orbit, it appeared that the effect of epinephrine and muscarine was not due to their effect on smooth muscles of the nictitating membrane or secondary to changes in the cardiovascular system induced by these agents.

In pharmacological response, extraocular muscle of the cat behaves like chronically denervated mammalian muscle, amphibian and avian skeletal muscles, and the mammalian intrafusal muscle fibers of the muscle spindle. It is known that sympathomimetic amines evoke a contracture and a propagated conduction in chronically denervated skeletal muscle. Many postulates have been suggested to explain the stimulatory effect of sympathomimetic amines on skeletal muscle. According to Knejević and Miledi epinephrine has a dual action at the neuromuscular junction. In a rat phrenic nerve-diaphragm preparation, they showed that epinephrine caused an increase in frequency of miniature end-plate potentials (MEPP) due to increased transmitter release. This action of epinephrine is followed by depression of the muscle, which they explained as decreased excitability of the muscle membrane causing postsynaptic block. A similar depressant effect of epinephrine was also observed by us on isolated superior oblique extraocular muscle.

Another possible explanation of the effect...
of epinephrine may be postulated on the basis of Burn and Rand's hypothesis. If the motor nerve fibers to extraocular muscle also contain adrenergic fibers, then cholinergic agents like muscarine may cause release of epinephrine, which will cause contraction of the muscle by increasing transmitter release. In presence of atropine this effect of cholinergic agents would be blocked, but exogenous epinephrine could still produce an effect.

By analogy with denervated muscle on which epinephrine has a stimulatory effect, one may add a further postulate to explain its mode of action. Luco and Sanchez suggested that epinephrine probably acts on the same receptor where acetylcholine is acting whereas, according to Bowman and Rapely the effects of epinephrine on the muscle are secondary to its effect on carbohydrate metabolism, involving hexosephosphate and resulting in the removal of calcium from the membrane—the latter causing propagated action potential and contraction.

Thus there are many possible explanations for the effects of epinephrine and other autonomic agents, but none is quite conclusive. However, these effects are not unusual and, as Kuffler and Vaughan Williams have suggested for frog slow muscle, extraocular muscle may also have physiological characteristics somewhere between those of smooth muscle and twitch skeletal muscle.

The author wishes to thank Dr. Cedric M. Smith for his critical advice during this work and Dr. Klaus R. Unna for providing the facility to complete this work in his laboratories.

REFERENCES


