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ON THE MODE OF ACTION OF TUBOCURARINE

BY

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It is commonly believed that the neuromuscular blocking action of (+)-tubocurarine cannot be satisfactorily explained if ascribed to its relatively weak inhibition of cholinesterases (ChE). An alternative explanation, however, has been suggested on the basis of the ability of tubocurarine to shift the optimum substrate concentration of acetyl-ChE to higher values (Župančič, 1953). The aim of the present work was to obtain more data on the action of tubocurarine on acetyl-ChE and to find out whether or not the results might throw any light on the behaviour of curarized muscles and ganglia.

METHODS

ChE activity was determined electrometrically by continuous titration with 0.02M-NaOH; the *p*H varied from 7.4 to 7.7. Solutions or suspensions were made up with saline I (mammalian tissues) or saline II (leech tissue). The volume of the reaction mixture was 10 ml.

Enzyme Preparations.—The human red blood cells in citrated blood were washed three times with NaCl (0.9%), haemolysed with distilled water, and diluted with the reaction mixture to 50 times the original blood volume.

The gastrocnemius muscles of cats and rabbits were cut twice to sections 25 μ thick, weighed, washed twice in a tenfold volume of saline I (0.1505M-NaCl, 0.0026M-KCl, 0.0018M-$CaCl_2$). A weight of 1 g. of normal muscle or 0.5 g. of denervated muscle was suspended in 10 ml. of the reaction mixture.

The grey matter of rabbit cerebral cortex was removed with scissors, weighed, finely ground and suspended in 9 parts of saline I. These preparations show predominantly acetyl-ChE activity (at $pS=2$ the hydrolysis of butyrylcholine was 18% of that of acetylcholine). One ml. of the suspension was used in 10 ml. of the reaction mixture.

The frozen skin-muscle bag of leech was cut twice to sections 25 μ thick, weighed, washed four times with saline II (0.1146M-NaCl, 0.0026M-KCl, 0.0018M-$CaCl_2$) whereby the butyryl-ChE of the interstitial fluid was removed. The sections (0.6 g.) were suspended in 10 ml. of the reaction mixture.

Substrate and Inhibitor.—Acetylcholine chloride was used as substrate throughout the experiments; (+)-tubocurarine chloride was used in a $10^{-3.5}$ M concn. and added to the reaction mixture before the substrate.

The measurements were performed at 38° C., except for the leech tissue, where the temperature was 18° C.

RESULTS

The inhibition by tubocurarine of all the cholinesterases used in this investigation is weak, and high concentrations must be used. However, a shift of the optimum substrate concentrations, toward higher values, was always found (Figs. 1–4).

DISCUSSION

In previous papers one of us (Župančič, 1953, 1955) proposed the hypothesis of receptor enzymes. According to this view, the receptors for various biologically active substances are assumed to be identical with more or less specific enzymes inactivating the particular substance. Thus the receptor protein for acetylcholine may be identical with tissue cholinesterase. Nevertheless it was felt that this view could hardly account for the behaviour of a family of agents of which tubocurarine is the classic representative; these substances are rather weak inhibitors of cholinesterase, but potent blocking agents of synaptic transmission. A closer examination, however, shows that this discrepancy is only superficial: from the pharmacological effects of such substances as stable choline esters, choline itself, nicotine or tetramethyl ammonium, it was concluded that acetylcholine acts at the moment when it is attached to the receptor cholinesterase and not at the moment of its hydrolysis. In neuromuscular transmission, acetylcholine thus causes quicker movements of ions across the post-synaptic membrane. The end-plate potential is thus set up while the cationic heads of its molecules are adsorbed to the anionic centres of receptor acetylcholinesterase. The hydrolysis by the esteroclastic centres only makes possible the repolarization of the membrane whereby the cycle can be repeated. The quaternary nitrogens of tubocurarine mole-

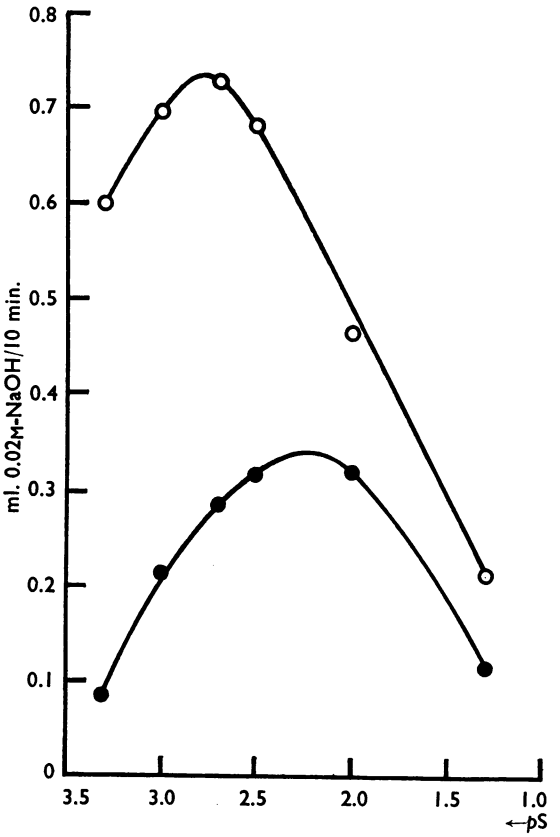

FIG. 1.—The hydrolysis of acetylcholine by human red blood cells in the presence (●) and absence (○) of $10^{-3.5}$ M-(+)-tubocurarine.

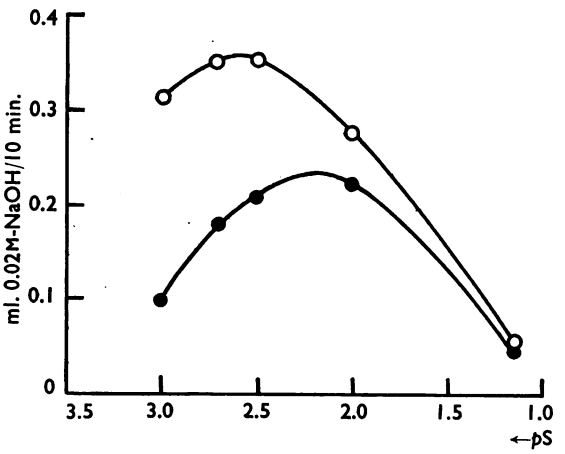

FIG. 2.—The hydrolysis of acetylcholine by cat gastrocnemius muscle in the presence (●) and absence (○) of $10^{-3.5}$ M-(+)-tubocurarine.

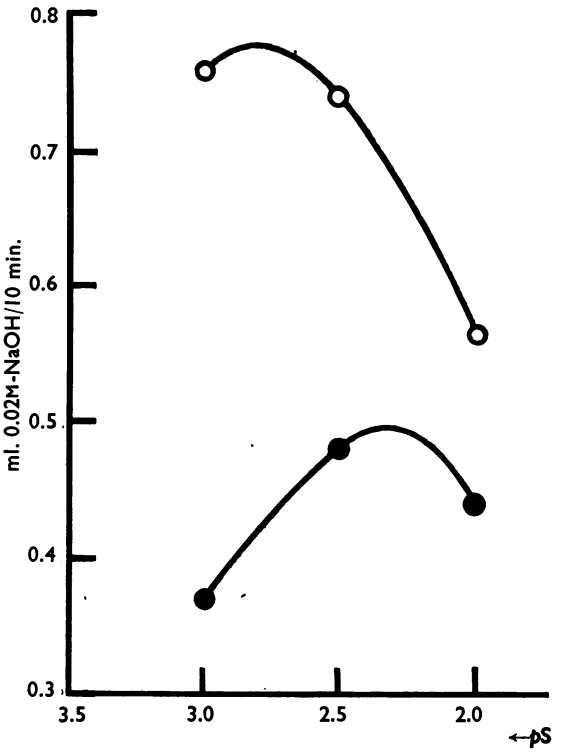

FIG. 3.—The hydrolysis of acetylcholine by rabbit cerebral cortex in the presence (●) and absence (○) of $10^{-3.5}$ M-(+)-tubocurarine.

cules thus compete with the cationic heads of acetylcholine for anionic centres of receptive cholinesterase without directly blocking the esteroclastic sites. Hence, with more and more anionic sites occupied by tubocurarine, the amplitude of the end-plate potential should decrease, and the whole process should eventually result in neuromuscular block as well as insensitivity to pharmacological acetylcholine. In the kinetics of acetylcholinesterase the theoretical effect of competition for anionic sites need not necessarily be inhibition of the hydrolysis, but rather prevention of inhibition by excess substrate—that is, a shift of the optimum substrate concentration to higher values (Augustinsson, 1948; Austin and Berry, 1953; Berry, 1953). With many substances even an activation of hydrolysis at higher substrate concentration has been shown. This is so, for example, with tetraethylammonium (Kensler and Elsner, 1951), a series of alcohols (Todrick, Fellowes, and Rutland, 1951), and with tubocurarine under certain conditions (Brzin and Župančič, 1955). In this laboratory a shift of the optimum substrate concentration accompanied by inhibition of activation of acetylcholinesterase was found for nicotine, hexa-

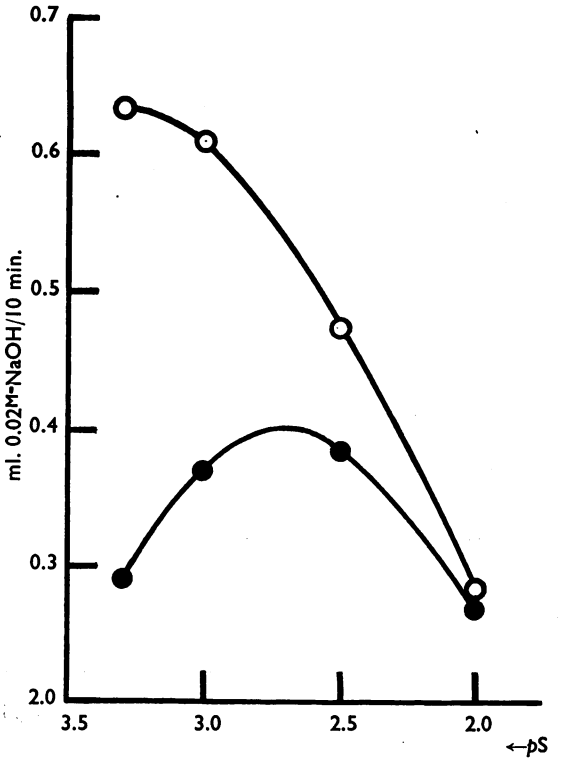

FIG. 4.—The hydrolysis of acetylcholine by leech skin-muscle bag in the presence (●) and absence (○) of $10^{-3.5}$ M-(+)-tubocurarine.

methonium, decamethonium and WIN 8077 (Kamarič and Župančič, 1956).

In our opinion, the so-called " direct " anticurare agents act by adsorption to the anionic sites of the receptive cholinesterase in the end-plate where they compete with tubocurarine.

On the other hand the " pure indirect " anticurare agents—such as dyflos or tetraethylpyrophosphate—acting through the esteroclastic sites of cholinesterase, do not shift the optimum substrate concentration (Augustinsson and Nachmansohn, 1949). It is clear that many substances can act at both the anionic and esteroclastic sites, and consequently display both the direct and indirect anticurare actions (e.g., neostigmine), the one or the other being prevalent.

With regard to the high concentration of tubocurarine used in this investigation the shift with curarization *in vivo* must be negligible. Yet it should be understood that the shift itself is not the cause of the synaptic block. It only represents evidence that tubocurarine combines with anionic sites of cholinesterase. In terms of the receptor-enzyme concept, the adsorption of a substance to the anionic centres of the receptive acetyl-cholinesterase logically calls for stimulation, or synaptic block, or both in succession.

SUMMARY

1. The influence of tubocurarine on the kinetics of 6 acetyl-cholinesterase preparations (human red blood cells, normal gastrocnemius muscle of cat, normal and denervated gastrocnemius muscle of rabbit, rabbit cerebral cortex, leech skin-muscle bag) was investigated.

2. Besides displaying a relatively weak inhibitory effect, tubocurarine produced a shift of the optimum substrate concentration towards higher values.

3. The significance of the results is discussed in terms of the receptor-enzyme concept.

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