Different Action of Atropine and Some Analogues on the End-Plate Potentials and Induced Acetylcholine Potentials

Atropine sulphate (AS) in concentrations lowering the amplitude of the end-plate potential (epp) by 50% markedly shortens the epp[1] and also shifts the point of reversal of the epp ($E_{r_{epp}}$) towards the Na equilibrium potential[2]. In order to estimate whether these effects of AS are due to a single mechanism, a study was made of the action of 7 tropine derivatives on the amplitude, shape and $E_{r_{epp}}$. Epp's were elicited by indirect stimulation (0.5 imp/sec) of the frog sartorius neuromuscular preparation (*Rana temporaria*) and registered by standard microelectrode technique (for details see[1]). High concentrations (8–12 mM) of $MgCl_2$ were used in the Ringer solution.

All the compounds studied lowered the epp and changed its shape after 15–20 min immersion in the muscle bath (Table). According to the effect on the shape, the compounds can be divided into 2 groups (Figure 1): (a) the AS type shortens both the rise- and decay-time; (b) scopolamine type: the rise-time is also shortened, but the decay phase has at least 2 components; the second part is markedly prolonged and the general picture resembles the effect of procaine[3,4]. Only the quaternary analogue of AS (methylatropine) and the tropine ester of diphenylacetic acid (tropacine) shift the $E_{r_{epp}}$ similarly as AS, whereas other related compounds (cocaine, tropacocaine) affect only the amplitude and shape of the epp without changing $E_{r_{epp}}$. Neostigmine sulphate (3×10^{-6} g/ml in the muscle bath) did not affect the $E_{r_{epp}}$ shift nor the changes in the epp shape. It is interesting that the shift of $E_{r_{epp}}$ begins to decrease after prolonged contact of the muscle with AS and after 4 h $E_{r_{epp}}$ approaches normal values.

In contrast to $E_{r_{epp}}$, $E_{r_{ACh}}$ (point of reversal of the potential evoked by iontophoretical application of the acetylcholine or butyrylcholine from double-barrel glass micropipette placed in the end-plate zone) was found to be the same in presence of AS (Figure 2) as in its absence in control experiments. This finding was rather unexpected, because only the quantitative differences between the activating effect of the natural mediator and ACh applied from a micropipette were determined as yet.

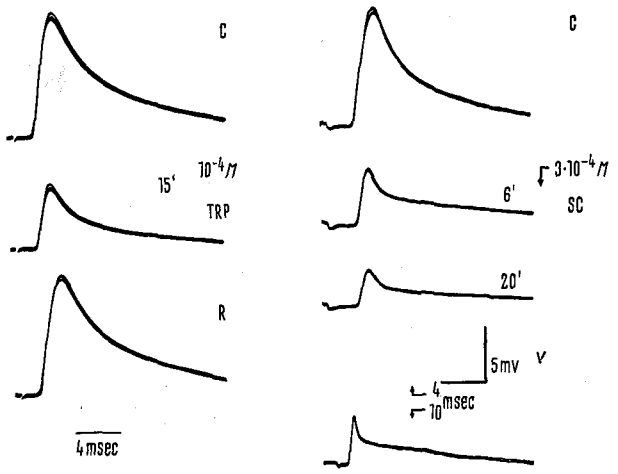

Fig. 1. End-plate potentials recorded intracellularly by glass microelectrodes (2.5 M KCl) from the end-plate zone of the muscle fibre (m. sartorius, *Rana temporaria*, April–July). Left-part, effect of tropacine. From top to bottom: C, control; $1\times10^{-4}M$ tropacine (TRP); R, recovery after 40 min. Right part: C, control; effect of scopolamine (SC, $3\times10^{-4}M$) after 6 and 20 min of its application in different time scales. 10.5 mM Mg^{++}, temperature 20 °C.

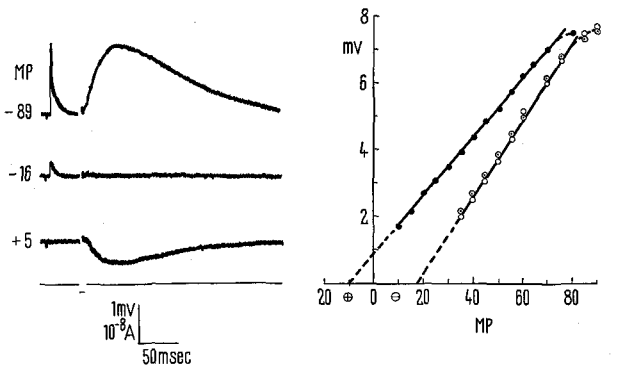

Fig. 2. Left part: subsequent oscillographic recordings of epp (first) and ACh potential (second) in the presence of $3\times10^{-5}M$ atropine at different levels of membrane potential (MP). The MP was lowered to the point of reversal (E_r) of ACh potential (– 16 mv) and to the E_r of epp (+ 5 mv) by a current, applied intracellularly from a second glass microelectrode (2.5 M KCl, resistance 5 M) placed about 100 μ from the recording one. Right part: another cell. ●, the dependence of amplitudes of simultaneously registered epp; ○, ACh potential and ⊙, butyrylcholine (BCh) potential on the membrane potential level in the presence of $3\times10^{-5}M$ atropine. E_r is determined by extrapolating the curves and is different for epp and ACh or BCh potentials respectively. Without anticholinesterase, temperature 20 °C.

Effect of some atropine analogues on the amplitude and shape of the epp's and on their point of reversal ($E_{r_{epp}}$)

	A_2	Changes in shape		$E_{r_{epp}} \pm$ S.E.
		Rise time %	Half time of decay %	mv
Control	–	100	100	-13.2 ± 2.5 (25)
Atropine	3.6×10^{-5}	60	60	$+9.2\pm4.1$ (15)
Methylatropine	5×10^{-5}	65	65	$+4.6\pm3.2$ (15)
Tropacine	1×10^{-4}	64	63	$+14.4\pm3.0$ (10)
Homatropine	5×10^{-4}	60	2-component form	-11.7 ± 4.0 (10)
Scopolamine	2×10^{-4}	61	2-component form	-13.5 ± 2.8 (8)
Cocaine	6×10^{-5}	64	60	-13.8 ± 3.4 (15)
Tropacocaine	4×10^{-4}	64	62	-12.9 ± 3.5 (14)

A_2, molar concentration of drugs causing 50% lowering of the epp amplitude. Figures in brackets indicate number of cells from which measurements were taken.

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The difference between $E_{r_{epp}}$ and $E_{r_{ACh}}$ that amounted to 23 mv cannot be explained by supposing that AS, methylatropine and tropacine influence the liberation of ACh from the nerve terminals, because E_r of the postsynaptic membrane is independent of the amount of mediator. Moreover, it is known that AS does not affect the quantum content of epp[1]. Also the existence of 2 cholinoreceptor types on the frog end-plate (muscarinic type connected with K^+ permeability, the inhibition of which by AS might cause a shift of $E_{r_{epp}}$, and the nicotinic type) was not confirmed[5]. Although it seems improbable, it is necessary to consider the possibility that apart from ACh, another different substance is liberated from the nerve terminal and that the action of this substance is affected selectively by AS, inducing a change in $E_{r_{epp}}$, whereas $E_{r_{ACh}}$ remains unchanged. However all present knowledge speaks against this hypothesis, including the fact, that the same concentration of AS lowers the epp and ACh potential in the same extent[1]. If we should assume, that AS hinders in some way the diffusion of K^+ from the synaptic gap, then we must expect according to TAKEUCHI[6,7] that the concentration of K^+ is approximately 50 mM in the gap, when E_r is shifted to + 9 mv. This is also improbable, because no depolarization of the presynaptic membrane (resulting in higher frequency of the miniature epp's) or decrease of the resting potential does not occur under AS. On the other hand, if we assume that Na^+/K^+ concentration in the synaptic gap does not change due to AS, then at $E_{r_{epp}}$ equal to + 9 mv (according to the formula presented by TAKEUCHI) $\Delta gNa/\Delta gK$ should give 2.6, which means it increases approximately twice. Such a change of the $\Delta gNa/\Delta gK$ ratio under the effect of atropine could explain not only its ability to block cholinoreceptors but also selectively block the potassium channel. However, even in this case it is difficult to explain the difference in the effect on the epp and the ACh-potential. It is of interest that the quaternary analogue of atropine, methylatropine, the molecule of which is fully ionized and therefore cannot easily enter the cell, has a similar effect to that of atropine[8]. The site of action of atropine and its analogues is apparently localized on the external surface of the muscle fibre membrane.

Changes in the shape of epp may also be the result of some postsynaptic processes, as can be concluded from our finding concerning the alteration of the shape of epp during artificial changes of the membrane potential. The epp thus reversed are distinctly 'faster' than normal epp (Figure 3). It is not possible to elucidate the change of shape of the epp by passive electric properties of the postsynaptic membrane, because the form of the postsynaptic current is influenced by atropine[9]. Moreover, a direct relationship between the form of transmembrane current and the corresponding course of the membrane potential was recently demonstrated[10].

Further analysis of the effect of atropine and its analogues on the postsynaptic membrane of the muscle fibre may throw light on the mechanism by which the interaction of the mediator with the specific receptor of the postsynaptic membrane gives rise to current flow across the membrane[11].

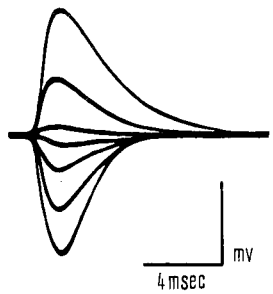

Fig. 3. Changes of the shape of epp at different values of MP (from − 40 to + 25 mv). The reversed epp's are markedly shortened. No drugs are present. Temperature 21 °C.

Zusammenfassung. Mittels intrazellulärer Mikroelektroden wurde der Einfluss von 7 Tropinestern auf Amplitude und Umkehrpunkt (E_r) der Endplattenpotentiale am M. sartorius des Frosches untersucht. Alle Substanzen erniedrigen die Amplitude und verändern die Form der Endplattenpotentiale, während 3 von ihnen den Umkehrpunkt (E_r) in der Richtung zum Na-Gleichgewicht verschieben.

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[11] This work was performed between February and July 1968.

Potentiation of Cutaneous Inhibition by Alcohol

Cutaneous nerves conducting corticopetal tactile sensibility from the body surface first synapse in the cuneate and gracile nuclei. Transmission across these nuclei shows a remarkable safety factor but is subject to strong presynaptic inhibition from cutaneous as well as contralateral cortical regions[1,2]. This inhibition functions as an important negative feedback mechanism controlling tactile sensory input at the level of the first central synapse. Central nervous system depressants, including

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