Some Characteristics of End-Plate Potentials After Partial Blockade by α-Bungarotoxin in *Rana temporaria*

Bungarotoxin (BuTX) and some other polypeptides from snake venoms are known to inhibit irreversibly the response of the postjunctional membrane elicited by nerve stimulation, i.e. end-plate potentials (EPPS). This effect is caused exclusively by the postsynaptic action of neurotoxins[1–3] which are supposed to react irreversibly with acetylcholine receptors[3–6]. If we consider the effect of toxins to be as specific as the effect of D-tubocurarine (DTC) and assume that the only difference is in the irreversibility or reversibility of the blockade, one can expect the properties like affinity to classical cholinolytics or reversal potential of EPPS, to remain unchanged after their partial blockade by both types of cholinolytics. The aim of the present report was to check this assumption.

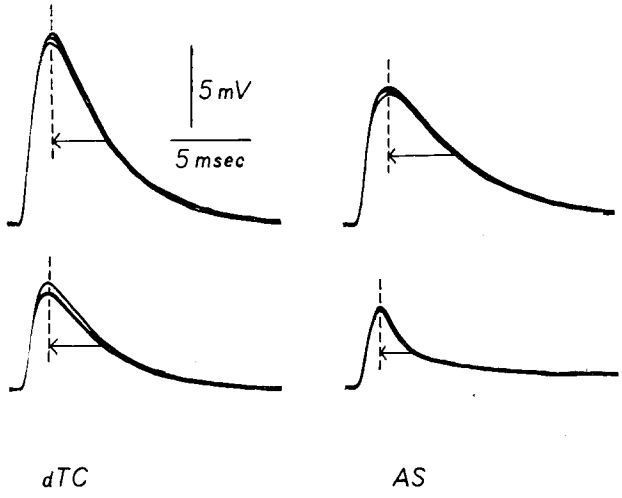

Fig. 1. Effect of D-tubocurarine (DTC) ($5\times10^{-7}M$) and atropine sulphate (AS) (3×10^{-5}) on the amplitude and time course of BuTX-treated end-plate potentials (3 superimposed records). Upper records, controls; lower records, after 20 min of DTC or AS action. Arrows indicate the half-time of EPP decay. Temperature 22 °C.

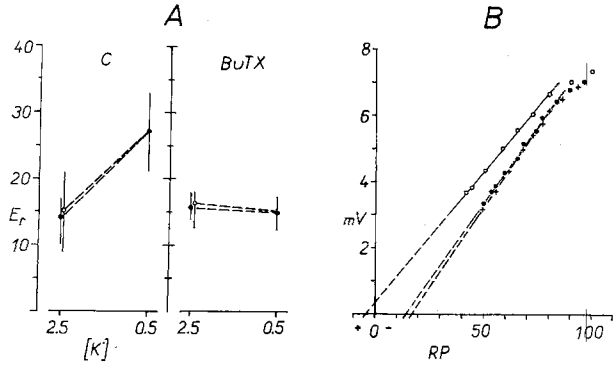

Fig. 2. Stabilization of E_r of BuTX-treated end-plate potentials (EPPS). A) Changes of E_r after diminishing of $[K^+]_o$ (in mM) and repeated increase to normal value (o) of the curarized ($5\times10^{-7}M$ DTC) (C) and BuTX-treated EPPS. Vertical bars are confidence limits. E_r is given in mV (negative). B) Effect of atropine sulphate on the E_r [cross section of resting potential (RP) with extrapolared lines of the EPPS (mV)] in the case of curarized (o) and BuTX-treated (+) EPP; ●, control before atropine.

Experiments were performed on isolated neuromuscular preparations of the frog sartorius muscle (*Rana temporaria*). Magnesium in the concentration of 10–12 mM was added to the muscle bath (mM: Na^+ 117; K^+ 2.5; Ca^{++} 1.8; Cl^- 120.6; HCO_3^- 2.4; pH = 7.4) and EPPS were registered by the conventional microelectrode technique. Another glass microelectrode (2.75 M KCl, 7–10 MΩ) introduced into the muscle fibre was used for artificial polarization of the membrane. BuTX (5×10^{-7} w/v in the bath) was usually allowed to act until the EPP amplitude decreased to 20–25% and the preparation was then washed with a solution containing only 6–7 mM; this procedure made it possible to work with EPPS of a sufficiently high amplitude (5–10 mV) on the background of lowered sensitivity, caused by the toxin.

The time course of EPPS was unchanged by BuTX, similarly as has already been found by LESTER[2] for Naja toxin. As can be seen from Figure 1, DTC and atropine sulphate (AS) in concentrations of $5\times10^{-7}M$ and 3×10^{-5} M respectively, exhibited the same potency and decreased the BuTX-treated EPPS to 50%[7]. AS also shortened the time course of EPPS in a typical manner[7–9] (Figure 1), whereas dTC somewhat shortened the rise time of BuTX-treated EPPS, having no effect on their decay time.

The linear relationship between the EPP amplitude and membrane potential was unchanged after BuTX action, the reversal potential of EPPS (E_r) being -16 ± 0.8 mV (9 experiments).

It is known that the electrogenesis at the postjunctional membrane possesses some specific features[9–13]. In particular, E_r of EPPS depends on the external concentration of potassium $[K^+]_o$. We studied the E_r of BuTX-treated EPPS after a change of $[K^+]_o$ from 2.5 mM (normal concentration) to 0.5 and again to 2.5 mM (Table I).

In control experiments with the curarized preparations, the E_r of EPPS was shifted regularly from normal values around −15 mV to about −28 mV when $[K^+]_o$ was diminished 5-fold which is in accordance with earlier observations[10]. But no such change was observed in the experiments with BuTX-treated EPPS (Table I, Figure 2A).

It has been formerly shown[14, 9] that atropine and some of its analogues shift E_r to the side of the Na equilibrium potential, i.e. to positive values. We checked this effect of AS

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on the BuTX-treated EPPS. AS in concentrations which diminishes the EPP amplitude to one half do not exhibit any effect on E_r (Table II, Figure 2B). This means that E_r of EPPS is 'stabilized' by BuTX and cannot be shifted either by $[K^+]_o$ or AS.

There are thus some properties of EPPS which remain unaffected by BuTX. These include the same affinity to classical cholinolytics, the time course of EPPS and its changes under AS and DTC which have the same pattern as in the controls without BuTX. But there exist pronounced changes in the electrogenic properties of the cholinoreceptive membrane after BuTX action which are not apparently connected so much with the state of surviving receptors as probably with ionophore.

Similar stabilization of ionophore action, i.e. disappearance of the E_r shift after diminishing $[K^+]_o$[13], or after AS (MAGAZANIK, VYSKOČIL, unpublished) was also observed at low temperatures (2–3 °C). Cold can hardly be thought to affect selectively the acetylcholine receptor only. The same doubts can also be expressed about the BuTX action. This polypeptide apparently not only blocks the receptors, but modifies the function of other links of the cholinergic transmembrane system, which are involved in electrogenesis at the postjunctional membrane.

Table I. Dependence of E_r of DTC (5 experiments) and BuTX-treated EPPS (11 experiments) on concentration of external potassium ($[K^+]_o$)

	K^+_o (mM)		
	2.5	0.05	2.5
E_rDTC	14 ± 1.4 (10.4 — 17.6)	27 ± 2.3 (21 — 33)	15 ± 2.0 (9 — 21)
E_rBuTX	16 ± 0.8 (14.1 — 17.9)	15 ± 1.2 (12.4 — 17.6)	16.5 ± 0.7 (14.9 — 18.0)

Numbers in brackets are confidence limits; P 0.05.

Table II. Effect of atropine sulphate ($3 \times 10^{-5} M$) on E_r of normal EPPS (E_r-N; 10 experiments) and BuTX-treated EPPS (E_r-BuTX; 15 experiments)

	Control		Atropine	
E_r-N	−16 ± 0.9	(14.1 — 17.9)	+8.0 ± 1.8	(4.1 — 11.9)
E_r-BuTX	−18 ± 2	(13.6 — 22.4)	−15 ± 1.0	(12.9 — 17.1)

Numbers in brackets are confidence limits; P 0.05.

Zusammenfassung. Nach Behandlung des Sartoriusmuskels von Rana temporaria mit BuTX wurden die Endplattenpotentiale durch Tubocurarin und Atropin mit unveränderter Wirksamkeit blockiert. Bungarotoxin stabilisiert das Umkehrpotential der Endplatte, welches nachher durch eine Verminderung der äusseren Konzentration von K^+ oder durch Atropin nicht mehr verändert werden kann.

L. G. MAGAZANIK and F. VYSKOČIL[15]

Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences, Leningrad (USSR), and Institute of Physiology Czechoslovak Academy of Sciences, Praha-4 (Czechoslovakia), 3 July 1972.

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Temperature Dependence of Naja Toxin Blocking Effect in *Rana temporaria*

The venoms of some snakes have recently been reported to contain polypeptides which possess great potency in blocking the chemosensitivity of skeletal muscles to acetylcholine irreversibly (ACh)[1–7]. The nature of interactions of these polypeptides with cholinergic membranes is not yet completely clear; it is assumed that their site of action is identical with that part of the receptor macromolecule, at which the primary reaction with ACh occurs. On the other hand, we have obtained some data[8,9] indicating that there does not exist a single ionic point at the muscle postjunctional membrane, which these polypeptides may occupy.

Temperature change is known to be a useful approach in the unravelling of biological and chemical mechanisms. In the present study we investigate the temperature dependence of Naja toxin (Naja TX) blocking effect with the aim of finding out whether simple ionic or more complicated interactions take place during its action on muscle end-plate potentials (EPPS). This toxin (polypeptide N3 isolated from the venom of *Naja naja siamensis*[10]) was preferred to a similarly acting α-bungarotoxin, because – as was found in several preliminary experiments – its blocking effect of EPPS is less variable in comparison with the latter.

All experiments were performed in vitro on the frog (*Rana temporaria*) neuromuscular preparation of sartorius muscle. Preparations were mounted in a translucide chamber with a Peltier semiconductor cooling device, which made it possible to maintain the temperature of the bath at required levels for sufficiently long periods of time. For intracellular recording of EPPS, neuromuscular transmission was partially blocked by adding $MgCl_2$ (10–12 mM) to the bathing Ringer solution (mM: Na^+ 117; K^+ 2.5; Ca^{++} 1.8; Cl^- 120.6; HCO_3 2.4; pH = 7.4). EPPS were registered from superficial muscle fibres by

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