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action, i.e. disappearance of the E_r shift after diminishing $[K^+]_o$[13], or after AS (MAGAZANIK, VYSKOČIL, unpublished) was also observed at low temperatures (2–3 °C).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

[1] C. C. CHANG and C. Y. LEE, Arch. int. Pharmacodyn. *144*, 241 (1963).
[2] C. Y. LEE, L. F. TSENG and T. H. CHIU, Nature, Lond. *215*, 1177 (1967).
[3] H. A. LESTER, Nature, Lond. *227*, 727 (1970).
[4] R. MILEDI and L. T. POTTER, Nature, Lond. *233*, 599 (1971).
[5] J.-P. CHANGEUX, M. KASAI and C. Y. LEE, Proc. natn. Acad. Sci., USA *67*, 1241 (1970).
[6] J.-P. CHANGEUX, J. C. MEUNIER and M. HUCHET, Molec. Pharmac. *7*, 538 (1971).
[7] R. MILEDI, P. MOLINOFF and L. T. POTTER, Nature, Lond. *229*, 554 (1971).
[8] L. G. MAGAZANIK and F. VYSKOČIL, Brain Res., in press (1972).
[9] F. VYSKOČIL and L. G. MAGAZANIK, Brain Res., in press (1972).
[10] E. KARLSSON, H. ARNBERG and D. EAKER, Eur. J. Biochem. *21*, 1 (1971).

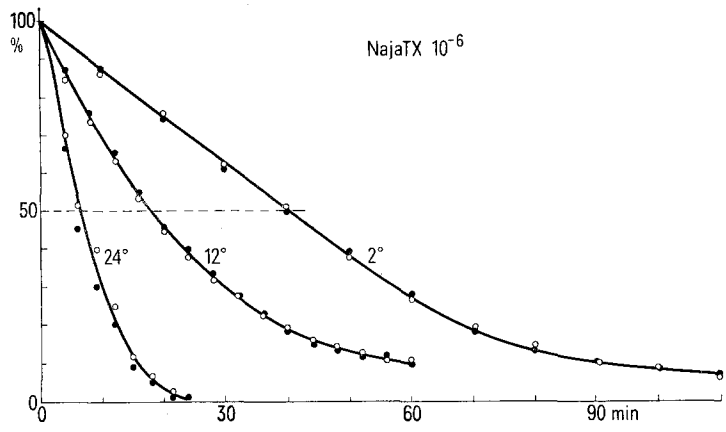

Fig. 1. Dependence of Naja TX blocking effect on temperature. Percentage decrease of EPPS amplitude was plotted against time. Separate registration of two EPPS were made from each muscle fibre at each temperature.

standard glass microelectrodes, monitored and photographed off an oscilloscope screen before and during Naja TX action. The freshly dissolved toxin in the final concentration of 10^{-6} v/w was used and the time course of its effect was measured at 24°, 12° and 2°C respectively.

At the temperature of 24°C (i.e. near room temperature) the Naja TX in the given concentration regularly decreased the EPPS amplitude with a half time of decay (T 1/2) of about 3–6 min (Table, Figure 1 A). Lowering the temperature to 12°C led to a decrease of the rate of toxin action which is reflected in marked prolongation of $T_{1/2}$; at 2°C the amplitude of EPPS of the surface fibres was only decreased during the first 20 min to about 75%. In contrast, practically no traces of EPPS can be found in superficial fibres of other muscles at around this time, when temperature of the bath was kept at 24°C. At all three temperatures, however, the percentage decrease of individual EPPS was surprisingly uniform (Table, Figure 1).

The mean values of $T_{1/2}$ were further used for estimation of the energy of activation of Naja TX binding at the post-junctional membrane. A graphical method was employed (Figure 2 A), when the ln of the reaction rate is plotted against the reciprocal values of absolute temperature[11].

The energy of activation (E) is then given by a simple formula $E = Tg\,\alpha \cdot R$ (R = gas constant). E of the Naja TX effect was found to be 3.4 kcal. mol. This value is sufficiently low to exclude the existence of a strong covalent type of binding of Naja TX at the membrane components. On the other hand, it is many times higher than the energy of a simple ionic interaction which is expected to underlie the ACh-receptor reaction[12, 13].

It is also known that the potency of the classical reversible cholinolytic agent, D-tubocurarine (DTC), does not depend to any great extent on the temperature[14].

We performed several experiments in which the blocking potency of DTC was measured under the same conditions as in the case of Naja TX, i.e. at 24°C, 12° and 2°C. As is shown in Figure 2 B, the potency of DTC to decrease the EPPS in 3 different concentrations is only little affected by temperature (10 experiments at each temperature). Comparing this fact with the great temperature dependence of Naja TX effect, one can postulate that the

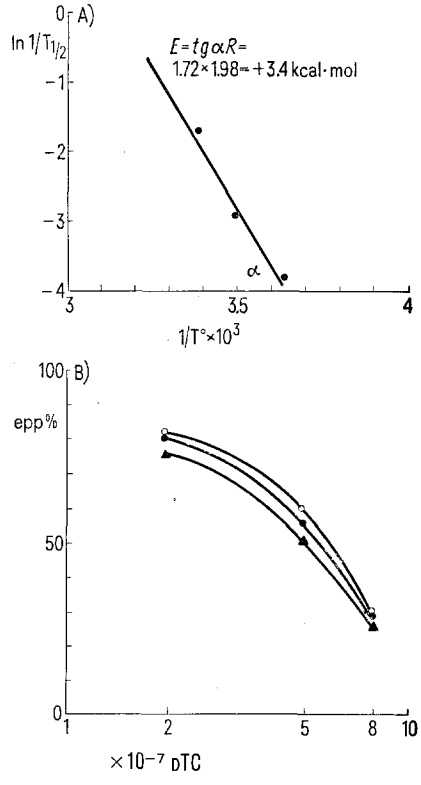

Fig. 2. A) Estimation of the energy of activation during Naja TX action. B) Temperature independence of D-tubocurarine (DTC) effect on EPPS amplitude. DTC was used at concentrations of 2,5 and $8 \times 10^{-7} M$ DTC. 0, 24°C; ●, 12°C; +, 2°C.

Temperature (°C)	$T_{1/2}$ of EPPS amplitude decay (min)
24°	3.0 ± 0.9 (0.7– 5.3)
12°	19.3 ± 0.7 (17.6–21.0)
2°	43.0 ± 1.7 (38.6–47.4)

Numbers in brackets are confidence limits. 10 EPPS were measured at each temperature.

[11] M. Dixon and E. C. Webb, *Enzymes* (Longmans, Green and Co, London 1958), p. 151.
[12] E. F. Van Maanen, J. Pharmac. *99*, 255 (1950).
[13] D. W. Jensen, Comp. Biochem. Physiol. *41*, 685 (1972).
[41] D. H. Jenkinson, J. Physiol., Lond. *152*, 309 (1960).

pattern of the toxin reaction and binding is more complex than the formation of the simple ionic pair of quaternal agents like DTC or acetylcholine.

It is highly probable that other types of linkage like hydrophobic, hydrogen bonds or van der Waals forces take place at the moment of functional failure of the cholinergic transmembrane system caused by contact with Naja TX.

Zusammenfassung. Die Abhängigkeit der blockierenden Wirkung des Najatoxins von der Temperatur zeigt, dass es bei seiner Einwirkung auf das cholinergische System nicht zur Bildung einer Ionenbindung kommt. Die Aktivierungsenergie der blockierenden Wirkung des Najatoxins war 3.4 kcal. mol.

F. VYSKOČIL and L. G. MAGAZANIK

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

Membrane Potential Measurement in Mouse Salivary Gland Cells

Membrane potentials in acinar cells have been measured in a number of different salivary glands from different species[1]. It has generally been found that the resting membrane potential (RP) is about –20 to –30 mV and that stimulation of the parasympathetic or sympathetic nerves to the gland causes a hyperpolarization. This stimulation-induced membrane potential change has been named the secretory potential[2]. The mechanism underlying the secretory potential has not been fully elucidated but different hypotheses have been proposed[2–4]. Since membrane potentials have not previously been measured in mouse salivary glands, this was attempted as a matter of routine, but since the results were surprising and illuminate some new aspects of salivary gland electrophysiology they are reported here.

Methods. The submaxillary or the parotid gland from young female mice was quickly removed after killing the animals and part of the gland was mounted in a perspex tissue bath through which a Krebs-Henseleit solution (37 °C, oxygenated) was pumped at a constant rate, as previously described for pancreatic preparations[5]. Membrane potentials were measured according to methods described by MATTHEWS[6] employing high resistance K-citrate filled micro-electrodes.

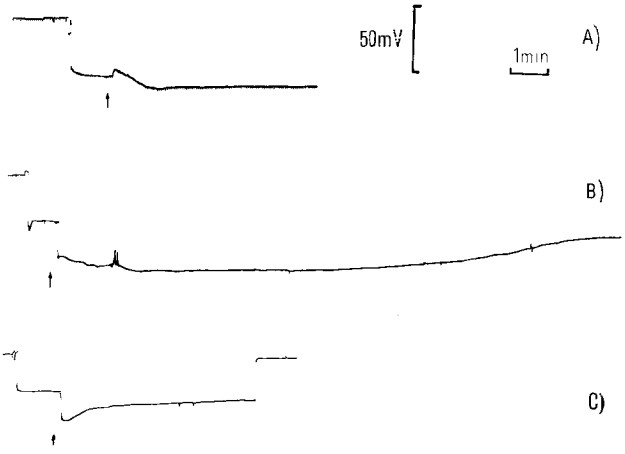

Fig. 1. Membrane potential measurement in mouse submaxillary gland. Downward deflections represent increased negativity of the micro-electrode. The sudden jump in potential seen in the left part of all 3 tracings corresponds in time to the insertion of the micro-electrode into the acinus. The arrows denote additions of ACh to the tissue bath to obtain for a short period a maximum concentration of 10^{-6} g/ml ($5.5 \times 10^{-6} M$). A and B were obtained during exposure of the gland to a normal Krebs-Henseleit solution while C was obtained during exposure to Strophanthin-G ($10^{-3} M$)-containing solution. B and C are from the same preparation.

Results. The resting membrane potentials in the mouse submaxillary gland ranged widely from –20 to –70 mV with a mean value of –47.0 mV ($n = 105$). In the parotid gland the mean value was –61.0 mV (52–70 mV) ($n = 20$). The micro-electrode was always inserted into cells situated just beneath the exposed surface of the gland. Figure 1 shows tracings of typical membrane potential recordings from the submaxillary gland. It is seen that acetylcholine (ACh) evoked biphasic secretory potentials. Sometimes a rapid shortlasting hyperpolarization was followed by a delayed longlasting hyperpolarization. At other times a rapid shortlasting depolarization was followed by a delayed longlasting hyperpolarization. When atropine sulphate ($1.4 \times 10^{-6} M$) was added to the superfusion fluid ACh failed to evoke any change in the membrane potential. In Figure 2 the dependence of the amplitude and polarity of the two phases of the secretory potential on the level of the resting membrane potential is shown. It is seen that the reversal potential for the rapid phase of the secretory potential was about –50 mV. During superfusion with a Strophanthin-G ($10^{-3} M$)-containing solution ACh always evoked monophasic hyperpolarizing secretory potentials of a relatively short duration (Figure 1). The mean value of the resting membrane potential (15–60 min after start of exposure to the drug) was –33.2 mV which was significantly ($P < 0.05$) lower than the resting membrane potential in the control periods of the same experiments.

Discussion. The very wide range of the magnitude of the resting membrane potentials in the mouse submaxillary gland is surprising but corresponds to a very recent finding in the cat submaxillary gland[7]. The fact that only cells very near to the surface were studied makes it highly unlikely that striated duct cells have been impaled. The secretory potentials obtained were similar to those seen in the acinar cells from the rat submaxillary gland[8]. It seems very likely, therefore, that the majority of the cells impaled were acinar, although high resting membrane potentials in salivary glands have previously been associated

[1] O. H. PETERSEN, Phil. Trans. R. Soc. Lond. B. *262*, 307 (1971).
[2] A. LUNDBERG, Physiol. Rev. *38*, 21 (1958).
[3] H. YOSHIMURA and Y. IMAI, Jap. J. Physiol. *17*, 280 (1967).
[4] O. H. PETERSEN, J. Physiol., Lond. *210*, 205 (1970).
[5] O. H. PETERSEN and E. K. MATTHEWS, Experientia *28*, 1037 (1972).
[6] E. K. MATTHEWS, J. Physiol., Lond. *189*, 139 (1967).
[7] A. NISHIYAMA and M. KAGAYAMA, Experientia, *29*, 161 1973.
[8] L. H. SCHNEYER and Y. YOSHIDA, Proc. Soc. exp. Biol. Med. *130*, 192 (1969).