Results. Under comparable conditions, the tumour tissue extracts had $1^1/_2$ to 2 times the protein content of the normal tissue extracts. The disc electrophoretic analysis revealed the difference to be mainly a quantitative one, mostly in the supernatants at 20,000 *g* (Figure 1). The specific activity of the tumour tissue isocitric dehydrogenase was about twice that of the normal tissue. Similar results were also obtained in the disc electrophoretic analysis. The isocitric dehydrogenase had a well defined pH optimum of 7.6, while consistently higher activity over the pH range 7.2–9.2 was obtained for the tumour tissue. The bulk of the isocitric dehydrogenase activity in both the tissues was localized in the supernatant at 20,000 *g*. There was an important qualitative difference between the 2 tissues with respect to the peroxidase isozymes (Figure 2.) This was further confirmed by the pH optima for peroxidase; being 5.2, 6.0 and 7.2 for the normal tissue and 5.2 and 6.4 for the tumour tissue.

Discussion. The higher isocitric dehydrogenase activity in the tumour tissue is quite significant, as MAINI, SRIVASTAVA and RAMAKRISHNAN[15] reported an NAD (NADP) glycohydrolase in the same tissue.

The present investigation has demonstrated quantitative (protein and -isocitric dehydrogenase) as well as qualitative (peroxidase) differences between the normal and the tumour tissue of *Rumex acetosa* maintained in vitro. The tumour specific characteristics are found to be transferred to the successive subcultures of the tissue indefinitely, and can be explained by the initial fixation of the information of the double stranded viral genome in the replicative DNA by the reverse transcriptase mechanism[6] which is transferred to the daughter cells via the daughter DNA and expressed as protein or isozyme characteristics even in the absence of the original virus[4,5], as observed in the present investigations.

Zusammenfassung. Es werden quantitative und qualitative tumorspezifische Merkmale von *Rumex acetosa* Geweben hinsichtlich Proteingehalt und Eigenschaften der NADP-abhängigen Isocitratdehydrogenase und der Peroxydas-Isoenzyme beschrieben. Das Weiterbestehen dieser Merkmale in den in vitro kultivierten Tumoren nach Verschwinden des Erreger-Virus wird auf die Aktivität einer reversen Transkriptase zurückgeführt.

A. S. SHIRODKAR and R. W. P. MASTER[16]

Department of Biochemistry, Wilson College, Bombay–7 (India), 31 January 1972.

[15] S. B. MAINI, S. K. SRIVASTAVA and C. V. RAMAKRISHNAN, Indian J. Biochem. *3*, 169 (1966).

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Depolarization by Acetylcholine (ACH Activation of Triphosphoinositide Phosphomonoesterase)

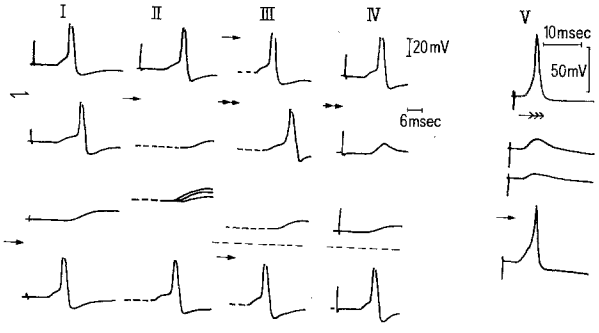
In cholinergic synapses, acetylcholine (ACH) generates postsynaptic depolarization by first forming a complex with its specific receptor located at the postsynaptic membrane. During this complex formation this specific receptor acquires the ability to initiate the processes that are needed for the execution of postsynaptic depolarization. The postsynaptic nicotinic receptor is the one involved in generation of fast bioelectric processes[1–4]. In in vitro biochemical experiments this nicotinic receptor was identified as the regulatory subunit of triphosphoinositide phosphomonoesterase (TPIPM)[5]. On combining with ACH, this regulatory subunit of TPIPM ceases to inhibit the enzymatic activity of the catalytic subunit of TPIPM and triphosphoinositide (TPI) becomes dephosphorylated to diphosphoinositide (DPI). This process initiates molecular reactions that control the local electric fields towards depolarization. The validity of the assumption that part of TPIPM functions as the specific nicotinic receptor of ACH at the postsynaptic membrane was here tested in vivo experiments.

Methods. The postsynaptic bioelectric processes of the superior cervical ganglion of the rabbit[6,7] were recorded with glass microelectrodes (0.5 μm tip diameter, 10 megaohm tip resistance, filled with 4 *M* K citrate), on a dual beam oscilloscope using a cathode ray follower. The presynaptic neuron was stimulated by constant voltage rectangular pulses (frequency 1/min) through platinum wire electrodes. At times hyperpolarizing currents were given through the intracellular microelectrodes. Intrapostsynaptic microinjections were given by combining pressure with iontophoresis (40 nA for 3–30 msec)[8]. The injected ACH, norepinephrine, and D-tubocurarine (DMTC) were commercially obtained, the subunits of TPIPM were prepared by the method described by TORDA[5]. 1 U of the catalytic subunit was arbitrarily chosen as the amount of enzyme that yielded 1 ng inorganic P/min. 1/2 U of the regulatory subunit caused 50% inhibition if incubated together with 1 U of the catalytic subunit. The subunits were injected in increasing amounts until bioelectric changes were observed. Less than 5 U always sufficed. Spontaneous recovery always occurred in less than 30 min.

Results. Near-synaptic microinjections of the catalytic subunit generated postsynaptic bioelectric processes (Figure) that were comparable to the effects of ACH (endogenous or exogenous). The endogenous ACH was released during presynaptic stimulation, the exogenous was delivered by near-synaptic microinjections. 1. The resting membrane resistance (aver. of 80 $\pm$ 1.8 mV) decreased in presence of subthreshold amounts of ACH or catalytic subunit. In both instances the equilibrium (reversal) potential averaged -7 mV. Postsynaptic EPSP and spiking did resemble. The average latency of EPSP was 2.8 $\pm$ 1.2 msec. The EPSP reached its summit (23 $\pm$ 2.4 mV) in an average of 4.4 $\pm$ 1.3 msec. The decay

[1] E. DE ROBERTIS, Science *171*, 963 (1971).
[2] S. EHRENPREIS, J. H. FLEISCH and T. W. MITTAG, Pharmac. Rev. *21*, 131 (1969).
[3] M. I. KABACHNIK, A. P. BRESTKIN, N. N. GODOVIKOV, M. J. MICHELSON, E. V. ROZENGART and V. I. ROZENGART, Pharmac. Rev. *22*, 355 (1971).
[4] N. V. KHOMEROV-BORISOV and M. J. MICHELSON, Pharmac. Rev. *18*, 1051 (1966).
[5] C. TORDA, Neurobiology *3*, 19 (1973); Fedn. Proc. *31*, 661 (1972); Biophys. J. *12*, 121 (1972).
[6] R. M. ECCLES and B. LIBET, J. Physiol., Lond. *157*, 484 (1961).
[7] B. LIBET, Fedn Proc. *29*, 1945 (1970).
[8] D. R. CURTIS, in *Physical Techniques in Biological Research* Ed. W. L. NASTUK; Academic Press, N.Y. 1964), vol. 6, p. 144.

approximated an exponential curve, with a time constant averaging 14.3 ± 1.8 msec. Depolarization was completed in less than 30 msec, and was not followed by an afterpotential. Subthreshold stimuli generated EPSP with a height below 23 mV. The threshold for spike generation averaged 23 ± 2.1 mV. The spike height averaged 100 ± 2.6 mV, the overshoot averaged 13 mV. Peak was reached in 3 msec, the length averaged 6.1 ± 1.2 msec, and was followed by an afterpotential (average height of 5 mV). The postsynaptic effects of subthreshold concentrations of ACH and the catalytic subunit were additive. Inhibition of TPIPM-activity (e.g. by microinjections of the regulatory subunit) decreased the postsynaptic effect of either of the 2 agents. This inhibition seemed to result from a decreased activity of the nicotinic receptor, since the regulatory subunit did not seem to affect the effects of hyperpolarizing agents (e.g. intracellularly delivered hyperpolarizing current, norepinephrine) or muscarinic receptors (B fibers lack them).



Effect of various procedures on postsynaptic bioelectric processes. Column I: Additive effects of subthreshold stimuli (catalytic subunit and presynaptic pulse). Row 1: Control: Postsynaptic response to presynaptic supramaximal pulse. Arrow: Gradual decrease of stimulus intensity. Rows 2 and 3: Decrease of postsynaptic response. Full arrow: Near-synaptic injection of subthreshold amount of catalytic subunit. Row 4: Full postsynaptic depolarization. The additive effect suggests that the 2 types of stimuli had a cooperative effect on the same postsynaptic process, e.g. subthreshold amounts of catalytic subunit of TPIPM, together with the amount of activated TPIPM through the endogenous acetylcholine (released by the presynaptic pulse) sufficed to evoke a spike. – Column II: Postsynaptic depolarization generated by the catalytic subunit of TPIPM. Row 1: Control (Same as Column 1, Row 1). Full arrow: Suspension of presynaptic stimuli, injection of catalytic subunit. Rows 2–4: Increasing postsynaptic depolarization occurred (less than 5 U sufficed for full depolarization and spike formation). Column III: Inhibition by regulatory subunit of postsynaptic depolarization generated by the catalytic subunit of TPIPM. Row 1: Full arrow: Depolarization by injected catalytic subunit. Double arrow: Injection of regulatory subunit of TPIPM. Row 2 and 3: Inhibition of postsynaptic depolarization. Dotted line: 30 min recovery period. Full arrow: Injection of only the catalytic subunit of TPIPM. Row 4: Full postsynaptic depolarization, suggesting that the lack of response in rows 3 and 4 was not due to deterioration of the neuron. – Column IV: Inhibition by the regulatory subunit of postsynaptic depolarization generated by presynaptic supramaximal pulse. Row 1: Control (Same as Column I, Row 1). Double arrow: Injection of regulatory subunit of TPIPM. Rows 2 and 3: Inhibition of postsynaptic depolarization. Dotted line: 30 min recovery period. Row 4: Full postsynaptic depolarization. Column V: Effects of catalytic subunit and presynaptic pulse (endogenous acetylcholine) in curarized synapse on postsynaptic neuron. Row 1: Control (Same as Column I, Row 1). Triple arrow: Onset of injections of D-tubocurarine. Rows 2 and 3: Synaptic block. Full arrow: Injection of catalytic subunit of TPIPM. Row 4: Full postsynaptic depolarization. (It seems that the catalytic subunit supplied to the postsynaptic membrane the factor that was not produced in the curarized postsynaptic membrane (see text)).

DMTC blocked the effects of ACH, not those of the catalytic subunit. This difference probably resulted from the following mechanism: Postynaptic depolarization seems to depend on activation of the catalytic subunit during complex formation of ACH and receptor. This is prevented because: DMTC has a greater affinity to the common receptor than ACH, and 2. On formation of a DMTC-receptor (regulatory subunit) complex, the catalytic subunit does not regain its enzymatic activity[5], probably because of the large size of DMTC[9]. For these reasons ACH cannot activate TPIPM. On the other hand, the injected catalytic subunit supplies to the curarized synapse the process than cannot be formed in presence of DMTC, and depolarization continues to be possible.

Discussion and conclusions. These in vivo experiments support the biochemical (in vitro) observetions[5] that the regulatory subunit of TPIPM is (one of) the specific nicotinic receptor (s) of ACH. Activation by ACH of TPIPM seems to be one of the molecular mechanisms that may couple in time and space the formation of ACH-receptor complex and postsynaptic depolarization. On combination with ACH, this regulatory subunit ceases to inhibit the catalytic subunit, and TPI is dephosphorylated to DPI. Since DPI is a weaker chelating agent than TPI[10,11], the formation of DPI concurs with some loss of membrane-bound Ca^{++}. This loss modifies the local electric fields towards depolarization[12–14]. ACH-generated conformational changes[1,4], and ionophoresis (e.g. passive Na^+ and K^+ transport causing a change of the sums of the equilibrium potentials towards depolarization[15]) may cooperate in generation of postsynaptic depolarization. The time factors involved seem to meet the requirements of fast synaptic transmission. The shortest measured delay in chemically transmitting synapses was 0.2 msec. Formation of the ACH-receptor complex is practically diffusion-limited, and may occur in a fraction of 0.1 msec. Since activation of the catalytic subunit concurs with formation of the ACH-receptor complex, it does not require time. The turnover rate of TPI may be as fast as 14/sec[5].

Zusammenfassung. Azetylcholin scheint die postsynaptische Depolarisierung durch Aktivierung von TPIPM (Triphosphoinositid Phosphomonoesterase) herbeizuführen und der spezifische, nikotinartige Azetylcholin-Rezeptor scheint dabei die regulatorische Untereinheit von TPIPM zu sein. Die Verbindung von Azetylcholin mit TPIPM setzt eine katalytische Untereinheit frei, die Triphosphoinositid in Diphosphoinositid umwandelt und zur Depolarisierung führt.

CLARA TORDA[16]

Mount Sinai School of Medicine and Downstate Medical Center, 101 West 12 Street, New York (N.Y. 10011, USA), 3 November 1972.

[9] J. R. SMYTHIES, Int. Rev. Neurobiol. *14*, 233 (1971).
[10] R. M. C. DAWSON, Biochem. J. *97*, 234 (1965).
[11] H. S. HENDRICKSON and J. L. REINERTSEN, Biochem. biophys. Res. Commun. *44*, 1258 (1971).
[12] J. M. DIAMOND and E. M. WRIGHT, A. Rev. Physiol. *31*, 581 (1969).
[13] H. LECAR and R. NOSSAL, Biophys. J. *11*, 1068 (1971).
[14] K. L. MAGLEBY and C. F. STEVENS, J. Physiol., Lond. *223*, 151 (1972).
[15] A. L. HODGKIN and A. F. HUXLEY, J. Physiol., Lond. *116*, 449 (1952).
[16] Presented in part at the Annual Meeting of the Biophysical Society, Toronto, Canada, February 1972.