CYTOCHALASIN B INFLUENCE ON MEGAKARYOCYTE PATCH-CLAMP

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RESEARCH SUBJECT

Cytochalasins have been found in megakaryocytes and platelets (1), in intestines and other organs with smooth muscle cells (2), in kidney and other tight epithelia (3) where they modify numerous cellular functions related to DNA synthesis or fragmentation, contractile actin microfilaments, delivery of newly synthesized membrane proteins, activation of apical K^+ channels, control of exocytic events, cell volume regulation. Many such functions could carry a great weight, both in the etiopathogenetic and therapeutic aspects of cancer.

We have already prudently applied very low doses of Cytochalasin B (Cyt. B) on human spontaneous and animal experimental cancer.

Therefore we further studied some effects of Cytochalasins on the excellent target cells of megekaryocytes (4).

METHODS

Male Wistar rats, weighing 300–400 g, were anesthetized and bled to death. Femurs were removed and bone marrow driven out into a plastic dish, containing the following Ca-free saline solution (mM): 135 NaCl, 5 KCl, 1 $MgCl_2$, 10 D-glucose, 10 HEPES, and stored at 4°C until use (5). A petty fragment of the split bone marrow was transferred to the recording chamber, containing about 1 ml of the following external solution (5) (mM): 120 NaCl, 5 KCl, 10 $CaCl_2$, 1 $MgCl_2$, 10 D-glucose, 10 HEPES to final pH 7.3 at 25°C. The internal saline solution contained (5) (mM): 150 KCl, 1 $MgCl_2$, 10 HEPES to final pH 7.2 with KOH 1 M. The base of the recording chamber was coated in advance with adhesive polypeptide (cell-tak®, Collaborative Biomedical Products; Two Oak Park, Bedford, MA). After megakaryocyte settlement on the bottom, the chamber was mounted on the movable stage of an inverted microscope (Leitz DM IL),

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and permanently perfused with external saline solution at room temperature. Cytochalasin B (Sigma-Aldrich, Milano, Italy) 0.4 mM was added to the external saline solution to yield a 100 µM concentration. Patch pipettes were pulled from 1.5 mm capillary glass (Baxter, USA) in a two-stage vertical puller (Narishige, Japan); when filled with standard internal solution, the resistance was 2–6 Mς. The current and voltage were measure with a patch-clamp amplifier (Axopatch model 1D, Axon Instrument, USA), and were monitored with a storage oscilloscope (HM204-2, Germany). Signals were recorded and analyzed using a Pentium computer equipped with a Digidata 1200 data acquisition system and pCLAMP software (Axon Instrument, USA).

RESULTS

1) Megakaryocytes spontaneously settled were normally 23 ± 9 µm diameter;
2) V_m of megakaryocyte slowly declines following perfusion with Cyt. B (Figure 1);
3) When Cyt. B solution is injected very near megakaryocytes, V_m declines rapidly within 6 sec. after a short latency of 1 sec. A new, slow, similar decline begins within 11.25 sec., after which potential remains comfortably constant (Figure 2);
4) With a patch-pipette filled with Cyt. B solution, very large and irregular V-waves appear that soon and spontaneously fade (Figure 3).

CONCLUSIONS

Patch-clamp technique is a recent laboratory technique that gives precise intimate understanding of many cellular mechanisms.

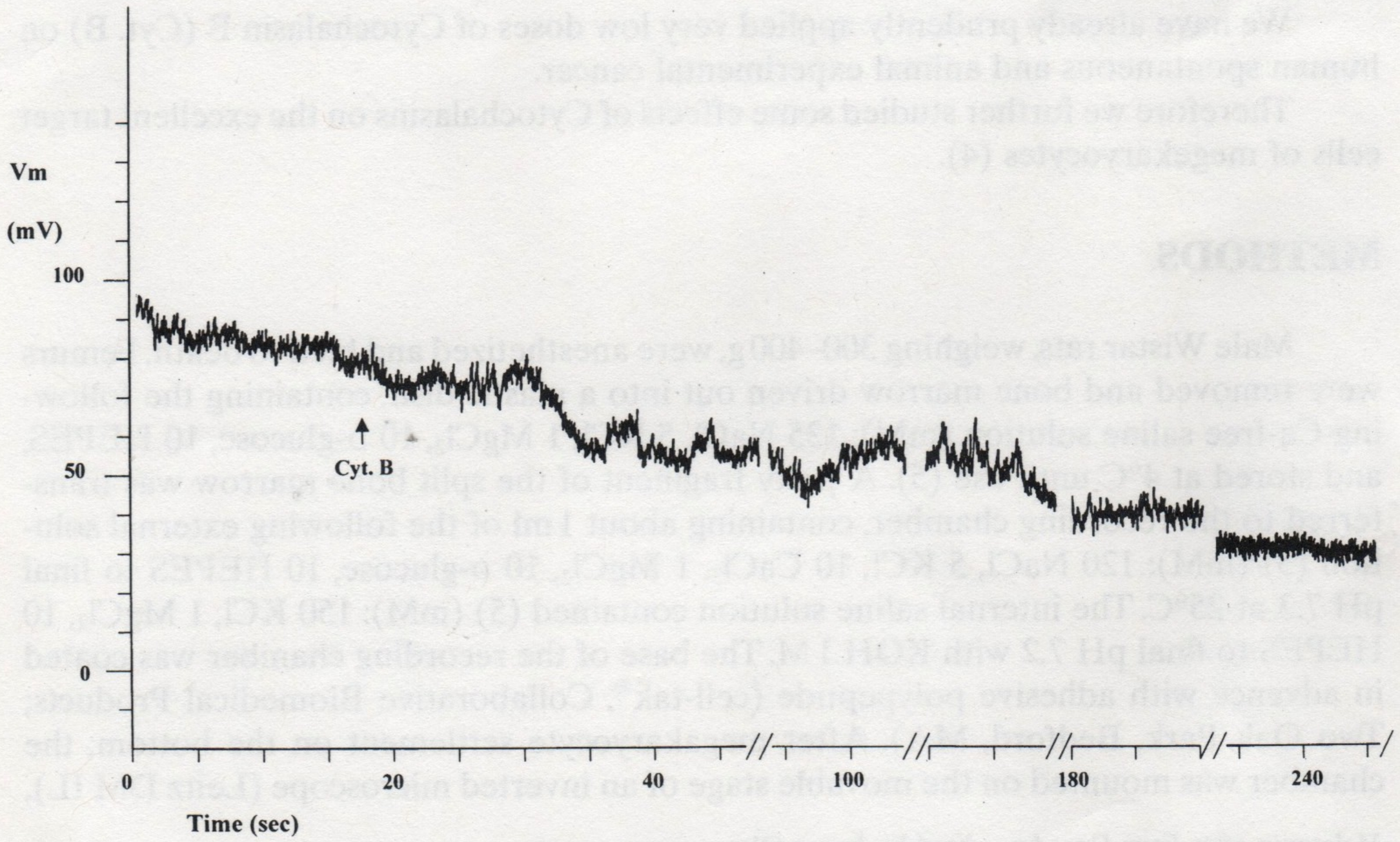

Figure 1. Megakaryocyte in cell-attached configuration. Following Cyt. B perfusing the Vm value declines.

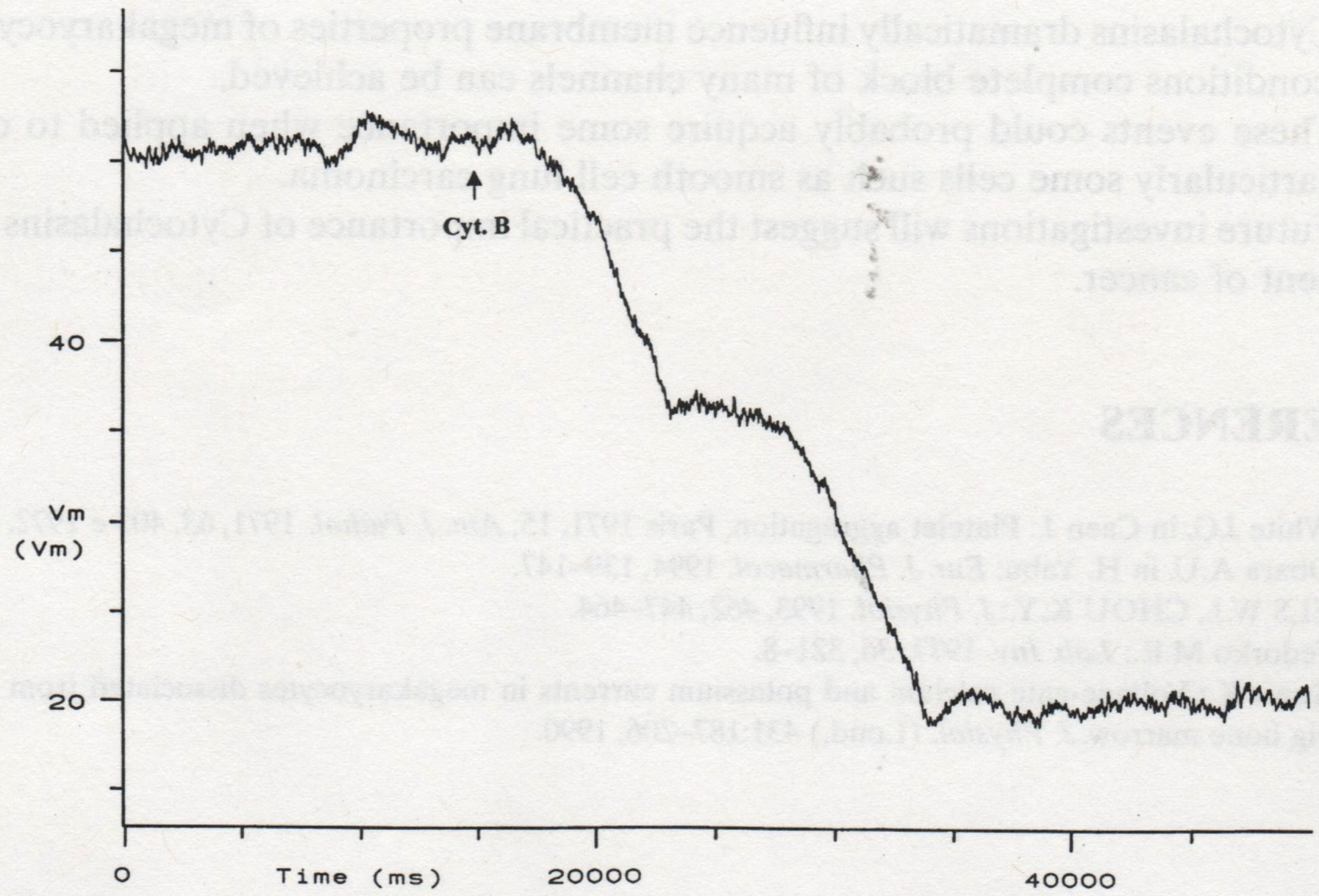

Figure 2. Megakaryocyte in cell-attached configuration. Cytochalasin B solution is injected near megakaryocytes at 20 sec. After a later time of about 1 sec. Vm declines by 50% within 6 sec. The decline stops after 8.5 sec. and is followed by a second almost identical decline by 50% (from +36mv to +18mV) after 11.25 sec. Vm then proceeds nearly stable.

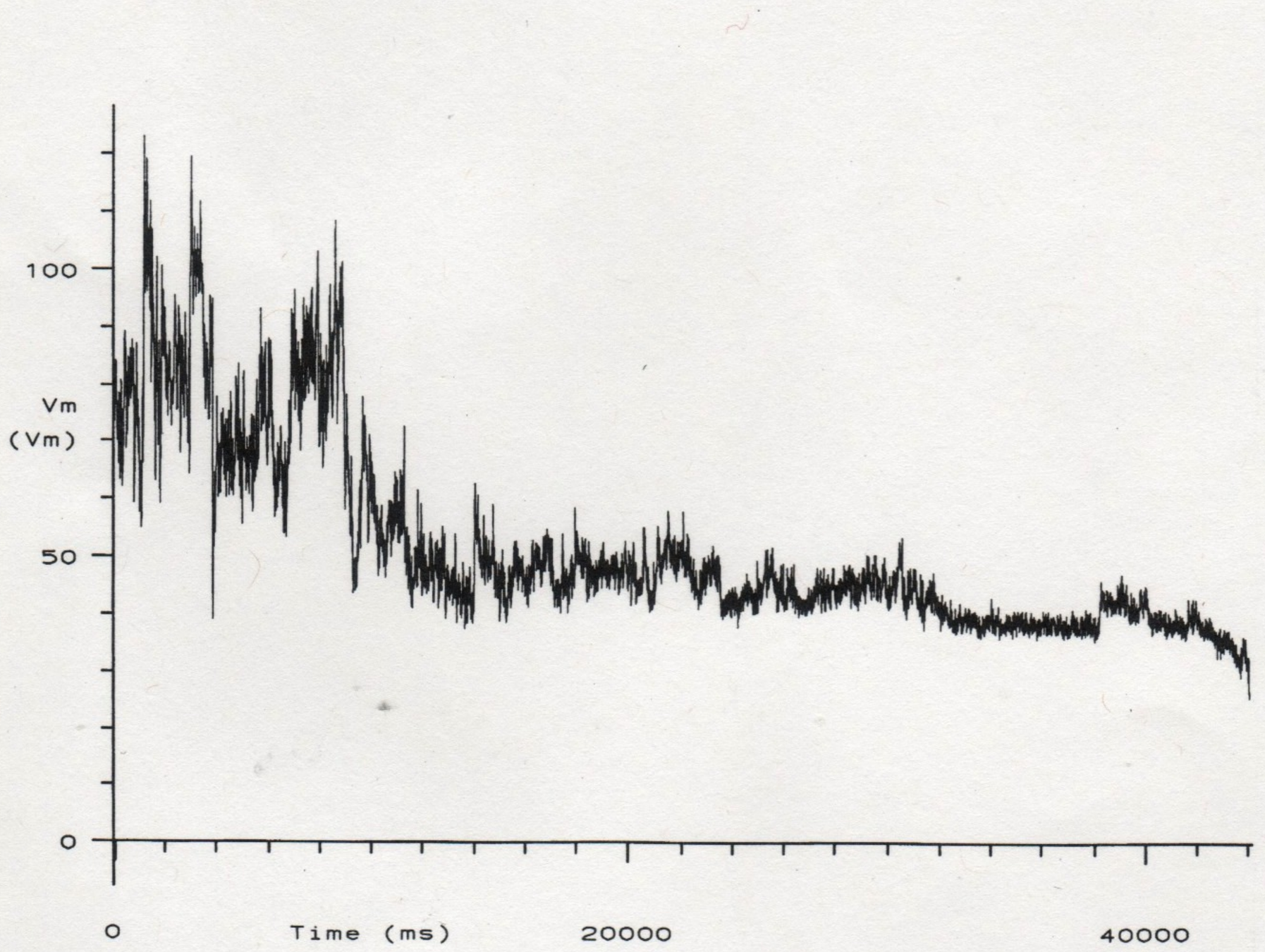

Figure 3. Megakaryocyte in cell-attached configuration. The Cyt. B solution perfusion was followed by a sprightly 7.6 sec. duration (about 50% intense decline) before gradually stabilizing at the lower level.

Cytochalasins dramatically influence membrane properties of megakaryocytes: in some conditions complete block of many channels can be achieved.

These events could probably acquire some importance when applied to cancer cells, particularly some cells such as smooth cell lung carcinoma.

Future investigations will suggest the practical importance of Cytochalasins in the treatment of cancer.

REFERENCES

1. White J.G. in Caen J.: Platelet aggregation. Paris 1971, 15; *Am. J. Pathol.* 1971, *63*, 403 e 1972, *66*, 295.
2. Obara A.U. in H. Yabu: *Eur. J. Pharmacol.* 1994, 139–147.
3. ELS W.J., CHOU K.Y.: *J. Physiol.* 1993, *462*, 447–464.
4. Fedorko M.E.: *Lab. Inv.* 1977, *36*, 321–8.
5. Kawa K.: Voltage-gate calcium and potassium currents in megakaryocytes dissociated from guinea-pig bone marrow. *J. Physiol.* (Lond.) 431:187–206, 1990.