EFFECT OF THE CALCIUM IONOPHORE A23187 ON NEURITE FORMATION BY NEUROBLASTOMA N2a CELLS

V. S. Gurevich, N. G. Potapova,
V. I. Ogurechnikov, and T. V. Ignasheva

UDC 612.119-08:612.419.014.2

KEY WORDS: neuroblastoma; calcium ionophore A23187.

The ability of neuroblastoma cells to undergo partial differentiation in culture makes them a convenient object with which to study molecular mechanisms of generation of excitation [5]. As the result of spontaneous or induced differentiation, neuroblasts can form electrically excitable outgrowths or neurites, which possess some of the properties of axons of mature neurons [7].

Neurite formation is stimulated, in particular, by agents controlling the level of cyclic nucleotides, the metabolism of which, in turn, is closely linked with calcium metabolism [6]. The role of calcium in processes coupled with the generation and conduction of excitation, of course, is well known [1].

Accordingly, in the investigation described below an attempt was made to study the effect of the calcium ionophore A23187, which, depending on concentration, acts both on the transport of Ca^{++} ions through the plasma membrane and on their intracellular translocation [3], on morphological differentiation of neuroblastoma cells.

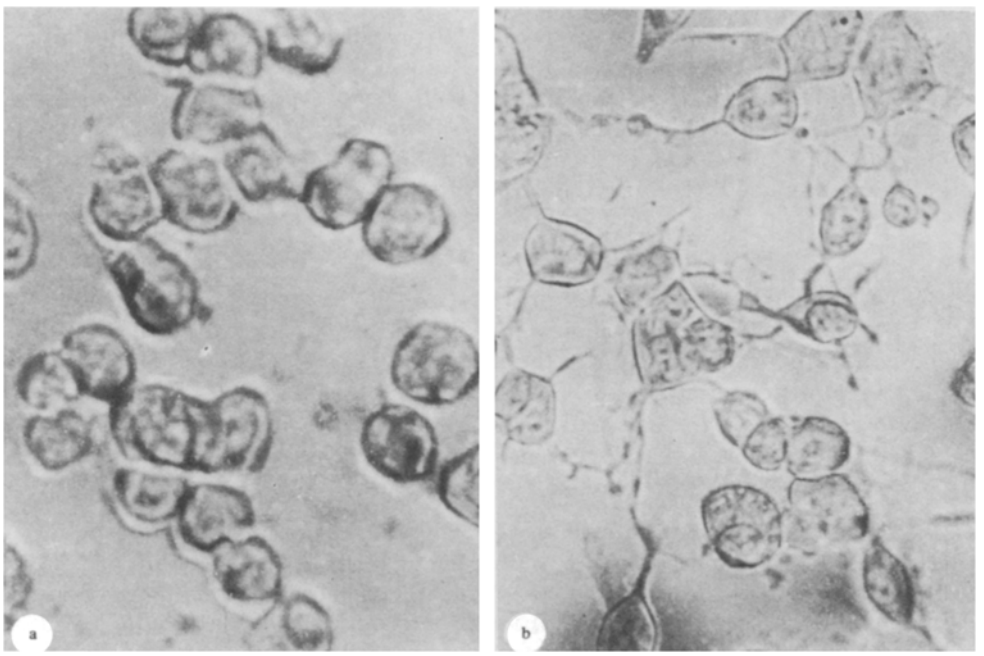

Fig. 1. Effect of ionophore A23187 on neurite formation by neuroblastoma N2a cells: a) control, b) 12 h after addition of 10^{-6} M A23187 to medium, 96 h of culture. 150×.

I. P. Pavlov Department of Physiology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 10, pp. 118-119, October, 1982. Original article submitted January 20, 1982.

0007-4888/82/9410-1455$07.50

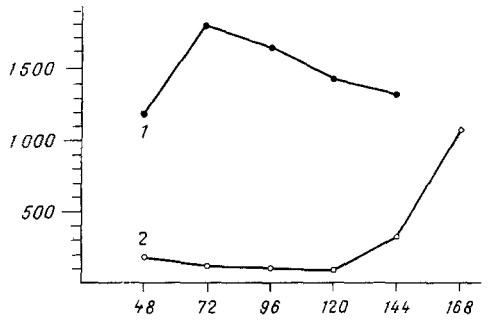

Fig. 2. DNA synthesis and $^{45}Ca^{++}$ uptake by neuroblastoma N2a cells during culture. Abscissa, time of culture (in h); ordinate, incorporation of label (in cpm/10^4 cells). 1) Incorporation of [^{3}H]thymidine, 2) $^{45}Ca^{++}$ uptake. Each point represents mean of four determinations.

EXPERIMENTAL METHOD

Cells of neuroblastoma N2a were maintained in a CO-22 CO_2 incubator (New Brunswick Co., Inc., USA) on plastic petri dishes 40 mm in diameter. The CO_2 concentration was 5%. The cells were cultured in Eagle's medium with the addition of 10% embryonic calf serum. The volume of medium was 2 ml. The seeding dose was 50,000 cells to 1 ml. Incorporation of [^{3}H]thymidine was determined by the method in [8]. The cells were incubated for 30 min at 37°C in the presence of 2 μCi [^{3}H]thymidine (specific activity 24 Ci/mmole), after which they were washed 3 times with cold 0.05 M Tris-HCl buffer, pH 7.4, treated with TCA, and allowed to stand overnight in 1 N KOH at 37°C. To measure radioactivity of the digest in a scintillation counter, a mixture of toluene, PPO, POPOP, and methylcellosolve was used. The cell concentration was determined in a Goryaev's counting chamber or from the protein concentration [4].

Incorporation of $^{45}Ca^{++}$ was investigated by the partially modified method described previously [3]. Cells were incubated in Eagle's medium containing 2 mM $^{45}Ca^{++}$ (specific activity 2.3 μCi/mmole). After 30 min the cells were washed with cold buffer and removed with a polyethylene loop; 0.2 ml of the suspension was transferred to vials for counting radioactivity, 0.5 ml of the tissue solvent BTS (from Beckman) was added, the mixture was incubated for 30 min at 55°C, and treated with 10 ml of toluene–PPO–POPOP mixture. A "Biostar" (USA) inverted microscope was used for intravital observation and photographing of the cells.

EXPERIMENTAL RESULTS

Ionophore A23187 was added in a concentration 10^{-4} or 10^{-6} M to neuroblastoma cells at different stages of culture. The A23187 was dissolved in methanol which, in the quantity used, did not affect the morphological characteristics of the cells. Addition of A23187 to the medium in a concentration of 10^{-6} M caused marked stimulation of morphological differentiation after 96-120 h in culture. The cells grew in size, became polygonal in shape, and up to 90% of them formed neurites (Fig. 1a, b). It must be emphasized that A23187 had no such effect when added to the medium at earlier stages of culture. This can be explained to some degree by the results of analysis of the dynamics of $^{45}Ca^{++}$ uptake by the cells.

As a result of spontaneous differentiation of the neuroblastoma the level of $^{45}Ca^{++}$ uptake did not rise until after 120 h in culture (Fig. 2), much later than the beginning of the fall in the rate of DNA synthesis, reflecting inhibition of proliferation of the culture. This fact is evidence that the triggering of morphological differentiation was not connected with intensification of the flow of extracellular calcium, and at the same time it indicates an increased demand of the nerve tissue for calcium for the realization of programs of biosynthesis for cell specialization. Tolerance of the neuroblastoma for the ionophore at earlier stages, in the writers' view, confirms this hypothesis indirectly.

The stimulating action of A23187 on neurite formation is dose-dependent. The optimal concentration of the ionophore was 10^{-6} M. With an increase in its concentration in the medium, neurite formation was not stimulated. In low concentrations A23187 is known to facilitate intracellular calcium translocation but not to affect its transport through the outer membrane [2]. It will also be evident that neurite formation is accompanied by

structural changes in intracellular membranes. It can accordingly be concluded that in this case the action of ionophore A23187 was mediated through intracellular mechanisms of the stabilizing effect of calcium on the membranes.

An essential contribution to the elucidation of this problem would be made by electrophysiological analysis of ionophore-treated cells. However, the experimental data described above indicate that the search for substances stimulating differentiation of nerve cells may be made among the specific effects of calcium metabolism.

LITERATURE CITED

1. S. Nemeček, Introduction to Neurobiology [in Russian], Prague (1978).
2. D. F. Babcock, N. L. Ferst, and H. A. Lardy, J. Biol. Chem., 251, 3881 (1976).
3. K. Kurzinger, C. Stadtkus, and B. Hamprecht, Eur. J. Biochem., 103, 597 (1980).
4. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 1265 (1951).
5. W. H. Moolenaar and I. Spector, J. Physiol. (London), 278, 265 (1978).
6. K. N. Prasad, Biol. Rev., 50, 129 (1975).
7. I. P. Ross, Tissue Cell, 7, 106 (1975).
8. M. M. Wick, Science, 199, 775 (1978).