TURNOVER OF OPIATE RECEPTORS IN NEUROBLASTOMA × GLIOMA HYBRID CELLS

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1. Introduction

Opiate receptors have been found in a neuroblastoma × glioma hybrid cell line [1,2] and have been shown to regulate adenylate cyclase [2,3] (ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1). The opiate receptor concentration is approximately twice that found in rat brain homogenates [1].

Morphological differentiation [4] and a concomitant increase in the specific activities of choline acetyltransferase (EC 2.3.1.6) and acetylcholinesterase (EC 3.1.1.7) [5] occurs in these cells when grown in the presence of dibutyryl cAMP. However regulation of the opiate receptor concentration has not been detected [1,6].

We report here the turnover rate of opiate receptors in NG108-15 neuroblastoma × glioma hybrid cells.

2. Materials and methods

NG108-15 neuroblastoma × glioma hybrid cells were grown in flasks (75 cm^2 surface area) at 37°C in growth medium containing 90% Dulbecco's modification of Eagle's minimum essential medium (GIBCO Cat. H-21); 10% fetal bovine serum; 0.1 mM hypoxanthine; 1 μM aminopterin and 12 μM thymidine. Cultures were maintained in a humidified atmosphere of 90% air and 10% CO_2. Care was taken to ensure that all test and control flasks were plated with closely approximating numbers of cells (8×10^5 cells/flask). The cells were used when judged to be about 40% confluent within the flasks.

Cycloheximide (Sigma Chemical Co.) was added to the test flasks at 20 μg/ml and the cells were harvested at intervals thereafter and washed 3 times with 10 ml Dulbecco's phosphate-buffered saline (without Ca^{2+} and Mg^{2+}) with centrifugation at 150 × *g* for 5 min after each wash. The cells from each plate were finally suspended in 1 ml 290 mM sucrose in 25 mM Tris–HCl, pH 7.4, and homogenized at 4°C with a Potter-Elvehjem homogenizer.

The binding of [^{3}H]naloxone was then assayed in triplicate in test homogenates and controls, to which no cycloheximide had been added. The binding assay was a modification of the method in [7]. Reaction mixtures of 200 μl contained the cell homogenate (150–420 μg protein); 130 mM sucrose; 12.5 mM Tris–HCl, pH 7.4; 100 mM sodium chloride and 60 nM [G-^{3}H]naloxone (17.2 Ci/mmol, New England Nuclear). Similar incubations were also performed in triplicate in the presence of 10 μM unlabelled naloxone (Endo Labs). Reaction mixtures were maintained at 4°C in an ice bath for 40 min, then filtered [7]. Each GF-B Whatman glass filter disc (24 mm diam.) was shaken for 10 min with 1 ml 1% (w/v) sodium dodecyl sulphate and then 10 ml Aquasol (New England Nuclear) was added prior to liquid scintillation spectrometry. Specific binding was calculated as the difference in binding in the presence and absence of 10 μM unlabelled ligand.

In another series of control and test flasks, L-[4,5-^{3}H]leucine (5 Ci/mmol, New England Nuclear) was added to the culture medium with spec. act. 0.2 μCi/ml and final conc. 0.8 mM. Cells from each flask were harvested and washed at selected times as described above, then dissolved in 4 ml 200 mM NaOH. After neutralizing with 1 M HCl, 5 ml 10% (w/v) trichloroacetic acid was added. The protein precipitate was washed 3 times at 4°C in 10 ml 5% (w/v) trichloroacetic acid with centrifugation after each wash at 30 000 × *g* for 30 min. Each pellet was

finally dissolved in 1 ml 1% (w/v) sodium dodecyl sulphate and 10 ml Aquasol was added.

The protein content of each flask was determined by the method [8], with re-crystallized bovine serum albumin as a standard.

3. Results and discussion

Culture of neuroblastoma X glioma hybrid cells in the presence of 20 μg/ml cycloheximide for periods longer than 24 h during their logarithmic growth phase resulted in cell death as measured by the permeability of cells to trypan blue dye. However less than 5% cell death occurred during the first 18 h. Cycloheximide was shown to inhibit protein synthesis at 20 μg/ml; [^{3}H]leucine incorporation into protein was reduced by over 90% over a period of 15 h. The effect of cycloheximide on opiate receptors and cellular protein per flask with respect to time is shown in fig.1. The opiate receptor concentration per mg cell protein was only slightly reduced by cycloheximide and is shown in fig.2.

The opiate receptors and protein content per flask decreased at similar rates (fig.3). A 50% reduction in protein content occurred in 19.5 h while a corresponding reduction in opiate receptors occurred in 16.5 h. The first order rate constant (k) for the decline in opiate receptors is 4.2×10^{-2} h^{-1}.

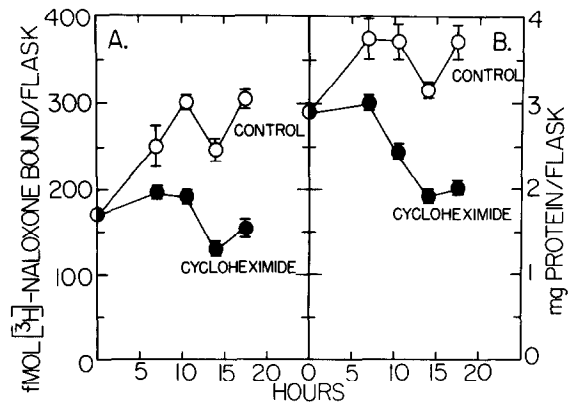

Fig.1. The effect of 20 μg/ml cycloheximide on NG108-15 hybrid cells, showing the changes in (A) specific [^{3}H]naloxone binding and (B) cellular protein. Results show the mean (±SEM) of triplicate flasks in the presence (•) and absence (○) of cycloheximide.

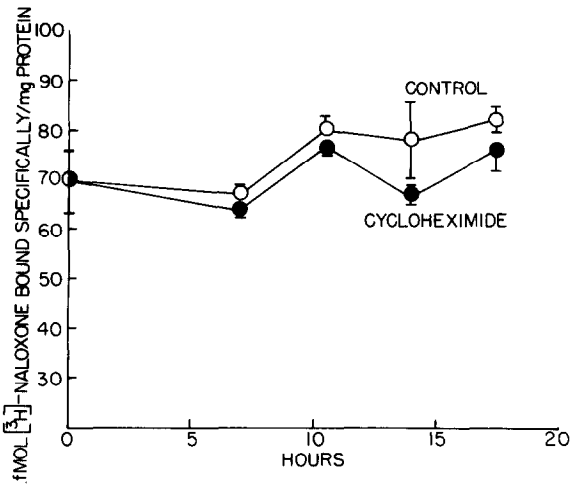

Fig.2. Opiate receptor concentration in NG108-15 hybrid cells, cultured in the presence (•) and absence (○) of 20 μg/ml cycloheximide. Results show the mean (±SEM) of triplicate flasks.

These results show that opiate receptors turn over at a rate similar to that of total cell protein. Culture of NG108-15 neuroblastoma X glioma hybrid cells in the presence of morphine does not alter the opiate receptor concentration [6], nor has desensitization of

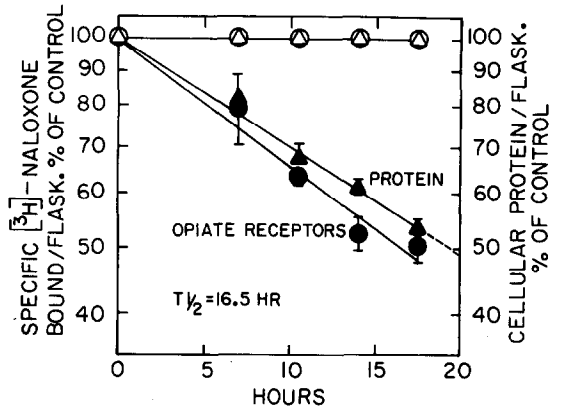

Fig.3. The decrease in both [^{3}H]naloxone binding (•) and cellular protein content (▲) in hybrid cells cultured in the presence and absence of 20 μg/ml cycloheximide. The result in each case is expressed as % control value (open circles and triangles). Each point shows the mean (±SEM) of triplicate flasks.

the receptor been detected with respect to inhibition of adenylate cyclase.

Little is known about the turnover rates of putative neurotransmitter receptors with the exception of nicotinic acetylcholine receptors in mouse muscle [9,10] and the muscarinic acetylcholine receptors of NG108-15 cells. The latter, which are also coupled to the inhibition of adenylate cyclase, turn over with a half-life of approx. 10.8 h [11] and at an increased rate in the presence of carbamylcholine [12].

References

[1] Klee, W. A. and Nirenberg, M. (1974) Proc. Natl. Acad. Sci. USA 71, 3474–3477.
[2] Traber, J., Fisher, K., Latzin, S. and Hamprecht, B. (1975) Nature 253, 120–122.
[3] Sharma, S. K., Nirenberg, M. and Klee, W. A. (1975) Proc. Natl. Acad. Sci. USA 72, 590–594.
[4] Daniels, M. P. and Hamprecht, B. (1974) J. Cell Biol. 63, 691–699.
[5] Hamprecht, B., Amano, T. and Nirenberg, M. (1978) in preparation.
[6] Sharma, S. K., Klee, W. A. and Nirenberg, M. (1975) Proc. Natl. Acad. Sci. USA 72, 3092–3096.
[7] Pert, C. B. and Snyder, S. H. (1973) Proc. Natl. Acad. Sci. USA 70, 2243–2247.
[8] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, P. J. (1951) J. Biol. Chem. 193, 265–275.
[9] Hogan, P. G., Marshall, J. M. and Hall, Z. W. (1976) Nature 261, 328–330.
[10] Brown, T. H., Noble, M. and Peacock, J. H. (1977) Ann. Meet. Soc. Neurosci. 3, 523 (Abstract).
[11] Klein, W. L., Nathanson, N. M. and Nirenberg, M. (1978) in preparation.
[12] Klein, W. L., Nathanson, N. M. and Nirenberg, M. (1976) Fed. Proc. Fed. Am. Soc. Exp. Biol. 35, 1576.