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Transduction signals induced in rat brain cortex astrocytes by the HIV-1 gp120 glycoprotein

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Abstract Cultures of rat brain cortex astrocytes were exposed to 10^{-10}–10^{-9} M of the HIV-1 envelope glycoprotein, gp120. No specific binding was revealed by the iodinated protein, suggesting expression of only a few sites onto the cells. In contrast, two transduction signals were rapidly induced by gp120: increased tyrosine phosphorylation of a ~56 kDa protein and increased $[Ca^{2+}]_i$. This latter effect, present in 1/3 of the investigated astrocytes, consisted in: discrete or biphasic peaks; slowly rising plateaus; and various types of oscillations. Moreover, in apparently unresponsive cells $[Ca^{2+}]_i$ rose slowly (45 min) to double the resting levels. Rat brain cortex astrocytes thus appear highly sensitive to gp120. The induced array of signals might contribute to neurotoxicity during HIV infection.

Key words: Astrocyte; gp120; $[Ca^{2+}]_i$; Tyrosine phosphorylation; HIV neurotoxicity

1. Introduction

During the past several years, interest concerning the neurotoxic effects of HIV and the mechanisms of this viral action has increased considerably. Initially, the effects were believed to be due to a direct action on the neurons, which however has never been clearly documented. As a consequence, growing attention was devoted to glial cells, especially to microglia that can be infected by the virus and thus release proteins of viral origin (see [1,2] and references therein). In our previous studies [3,4] on rat cerebellar cultures we demonstrated that one such protein, the env product gp120, when administered at concentrations as low as 10^{-10} M is able to induce distinct $[Ca^{2+}]_i$ responses in a considerable fraction (~50%) of the majority glial cells, the astrocytes, and also in oligodendrocytes. In contrast, no effect was observed in cerebellar granule neurons [4], a type of cell in which only indirect $[Ca^{2+}]_i$ responses to gp120 have been reported, mediated by depolarization and glutamate release [5,6]. Taken as a whole these results suggested gp120 to be (one of) the mediator(s) by which the delicate interactions among the various cellular components of the brain are affected in the course of HIV infection, with possible ultimate development towards direct neurotoxicity [4,6,7].

Although by themselves clear, our previous data on cerebellar glial cells [4] were limited. Because of their relatively small size, cerebellar cultures are hardly appropriate for biochemical experiments that require considerable amounts of material. Moreover, because of the specialization of the cerebellar cortex, the generality of the results was open to question. In order to deal with these aspects we have carried out new experiments in which the $[Ca^{2+}]_i$ responses were investigated in parallel to ^{125}I-gp120 binding and tyrosine phosphorylation in astrocyte cultures prepared from the rat brain cortex.

2. Materials and methods

Rat astrocytes were prepared from 2 day old rat cortex and cultured as described [8]. Before the $[Ca^{2+}]_i$ experiments the cultures were dissociated and the cells seeded onto glass slides [4]. Experiments were carried out on 2–7 DIV cultures. Immunocytochemical identification of the cells was carried out by using antibodies against the following markers: glial fibrillary acidic protein (astrocytes); A2B5 (type-2 astrocytes); galactocerebroside (oligodendrocytes, see [4] for details).

To reveal binding, gp120 was iodinated via two procedures: oxidation by lactoperoxidase and acylation by the Bolton-Hunter procedure. Control of the iodinated protein was achieved by radioautography of SDS-polyacrylamide gels. Experiments were carried out with either intact cell monolayers (bathed in Krebs-Ringer-Hepes buffer) or suspensions of membranes in 0.5 ml PBS (obtained from post-nuclear supernatants centrifuged at 40 000 rpm for 1 h), incubated for 1 h at 4 and 36°C, respectively. Concentrations of the label ranged from 20 and 2000 pM. To reveal unspecific binding parallel samples were supplemented with 10 nM unlabeled gp120 (for details see [9]).

For tyrosine phosphorylation, cell monolayers as above were exposed for 1–10 min at 37°C to gp120 in the 0.1–1 nM range. Additional experiments were carried out (0.5 nM gp120, 1 min) with cells either incubated in Ca^{2+}-free medium (with EGTA, 1 mM) or preloaded with the Ca^{2+} chelator, BAPTA (5 μM BAPTA-AM, 30 min) [10]. Incubations were stopped with 1 ml of SDS mixture, cells were scraped out, and samples boiled and run in SDS-polyacrylamide gels. Western blots [11] with anti-phosphotyrosine antibodies were decorated with ^{125}I-protein A and ^{125}I-radioautograms analyzed with a Molecular Dynamics Imagequant scanner.

Single cell $[Ca^{2+}]_i$ assays were carried out by imaging fura-2 loaded astrocytes as described in [4]. In all experiments, conditions were carefully established to avoid activation of cells by mechanical stimulation. At the end of the treatments the monolayers were always exposed to glutamate (5–100 μM) to check the general responsiveness of the cells.

2.1. Materials

The sources of most materials employed are indicated in [4]. Lactoperoxidase was obtained from Sigma, ^{125}I and Bolton-Hunter reagent from Amersham International and anti-phosphotyrosine antibody from Zimed.

3. Results and discussion

Binding experiments, carried out under the conditions specified in section 2, remained unsuccessful inasmuch as, at 0.02–2 nM gp120, i.e. at concentrations eliciting signal responses (see below), in no case was significant specific labeling appreciated above unspecific labeling. Since however the values of the latter were considerable, these negative results do not exclude the existence in astrocytes of specific high affinity binding sites for gp120, expressed however at levels (e.g. a few

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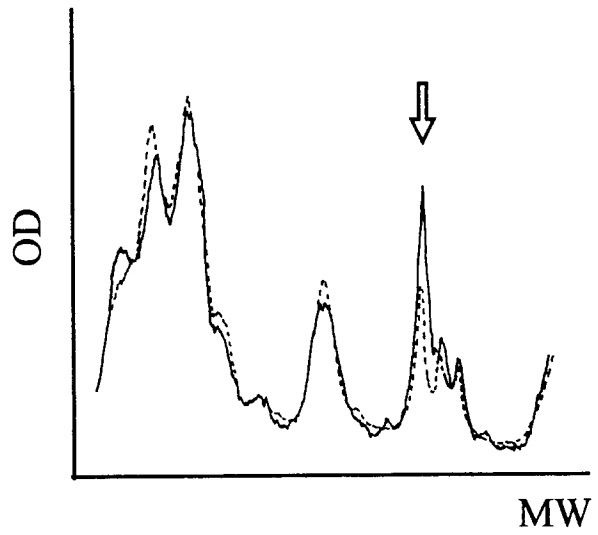

Fig. 1. Optical density profile (OD) of protein bands from rat brain cortex astrocyte cultures, separated by SDS-polyacrylamide gel electrophoresis and revealed by Western blotting. Controls are denoted by the dashed line, the samples treated for 1 min with 0.5 nM gp120 by the continuous line. The open arrow points to a band, ~56 kDa in apparent mol. wt.

hundreds or less/cell) distinctly lower than in lymphocytes where binding is easily appreciable.

Tyrosine phosphorylation was revealed by phosphotyrosine Western blotting in cultures treated with gp120 in the 0.1–1 nM range. A distinct signal, with increased phosphorylation of a band at ~56 kDa, appeared already 1 min after the application of the viral protein (Fig. 1) and remained appreciable thereafter. Such a result was not a consequence of the $[Ca^{2+}]_i$ increase concomitantly induced by gp120 ([4] and see below) since the response was changed neither when the experiments were carried out in the Ca^{2+}-free medium nor when

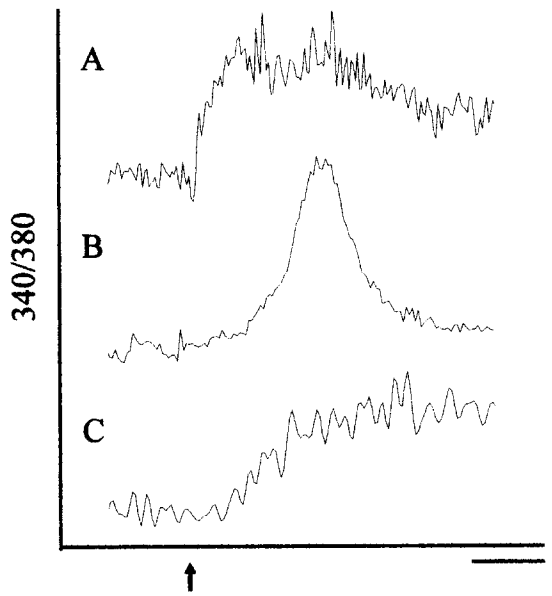

Fig. 2. Various types of $[Ca^{2+}]_i$ responses revealed by the fura-2 ratiometric (340/380) procedure in single cultured astrocytes exposed (arrow) to 0.8 nM gp120: a single and a biphasic peak (B,A); a slowly developing plateau (C). The bar at the bottom = 3 min.

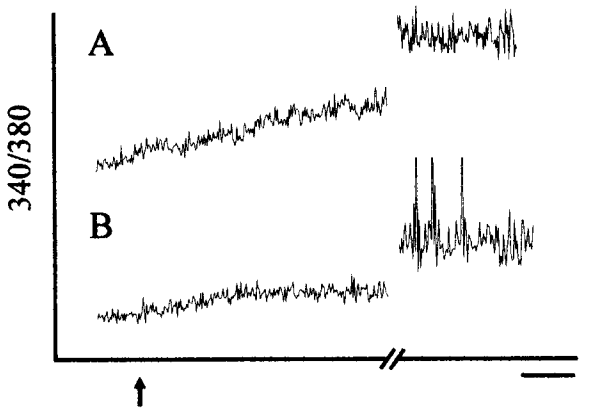

Fig. 3. $[Ca^{2+}]_i$ levels in single astrocytes that, in the few minutes after treatment with gp120 (0.8 nM, arrow), showed only small changes and that were therefore considered unresponsive to the viral protein. After a 45 min break in the recording (//), however, their $[Ca^{2+}]_i$ levels were found to be distinctly higher and in trace B also oscillating with respect to the levels at rest. The bar at the bottom = 3 min.

the cytosolic buffering of the cells was increased by their preloading with the Ca^{2+} chelator, BAPTA (see [10]). The alternative possibility, i.e. that the $[Ca^{2+}]_i$ responses are to be initiated by tyrosine phosphorylation, possibly via activation of phospholipase Cγ, remains to be investigated.

$[Ca^{2+}]_i$ imaging studies were carried out by the fura-2 ratio technique in cultured cells characterized in preliminary experiments according to the immunocytochemical criteria specified in [4]. In a total population of 300 astrocytes, those that responded to the application of gp120 (0.2–1 nM) with a rapidly appreciable increase of their $[Ca^{2+}]_i$ were 98 (33%). The responses, however, were quantitatively and kinetically different, as documented in Figs. 2–4. In the majority of the responsive cells $[Ca^{2+}]_i$ was stable before treatment (Figs. 2A–C and 4, top trace in A and both traces in B). The increases induced by gp120 were often discrete, consisting of either single peaks lasting 2–3 min; apparently biphasic responses, composed by an initial transient followed by a plateau; or slowly developing, persistent plateaus (Fig. 2A–C). In additional astrocytes, classified here as unresponsive, inappreciable or very small rises occurred during the first 5 min after gp120 application, yet $[Ca^{2+}]_i$ measurements 45 min later revealed clear increases, stable or oscillating, with respect to the resting levels (Fig. 3A,B). All analyzed cells (responsive and non-responsive to gp120) developed $[Ca^{2+}]_i$ spikes when treated with glutamate at the end of the experiments (not shown). This response confirms that exposure to the viral protein has no immediate toxic effects on the analyzed astrocytes.

A group of responsive astrocytes (~30%) exhibited $[Ca^{2+}]_i$ oscillations (Fig. 4). In some of these cells oscillations appeared only after gp120 administration, in others they were visible (although rare, small and irregular) already before treatment, and were converted by gp120 into higher and more frequently occurring spikes (top and bottom traces in Fig. 4A, respectively). The property of the viral protein to induce rapid oscillations (arranged in either bursts or trains persistent for tenths of min) became more evident when the Na^+ in the bathing medium was replaced by *N*-methylglucamine (Fig. 4B). More evident than in cerebellar astrocytes [4], this Na^+ replacement treatment induced by itself large, irregular, yet transient Ca^{2+} spikes. This result can be explained

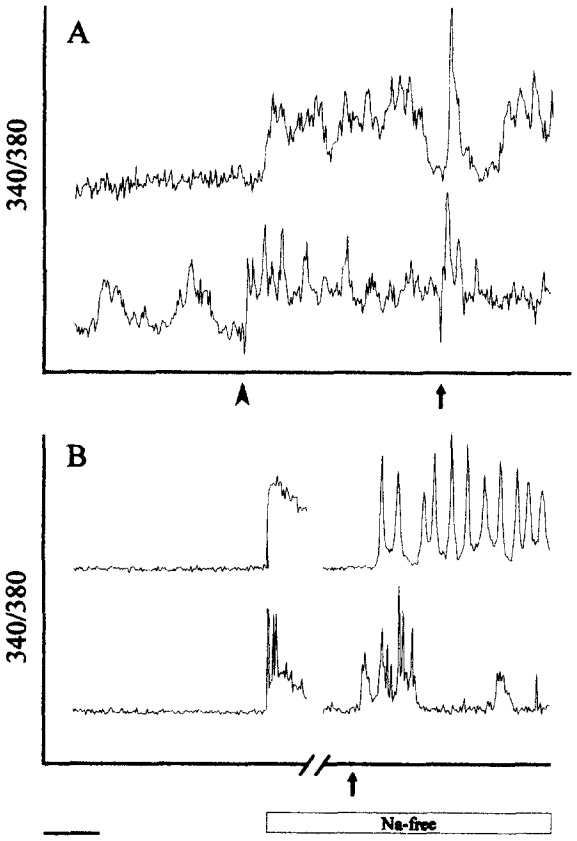

Fig. 4. $[Ca^{2+}]_i$ oscillations in single astrocytes exposed to gp120. (A) Two cells, incubated in Krebs-Ringer Hepes medium, where resting $[Ca^{2+}]_i$ was stable (top) or slowly oscillating (bottom). After administration of gp120 (0.2 and 0.8 nM at the arrowhead and arrow, respectively) irregular oscillations appeared in both. In the bottom trace they were distinctly more frequent than those visible at rest. (B) Two astrocytes where withdrawal of Na^+ from the medium induced by itself large and irregular, yet transient $[Ca^{2+}]_i$ spikes. After extinction of these spikes, the astrocytes responded to gp120 (0.8 nM, arrow) with appearance of long lasting oscillations, regular in the top, irregular in the bottom cell. The calibration bar represents 1.5 min in A and 3 min in B.

by the role of the Na^+/Ca^{2+} exchanger in the Ca^{2+} extrusion process [12]. Although variable in their kinetics, the $[Ca^{2+}]_i$ responses induced by the administration of gp120 might also be of importance because they could remain not necessarily confined to individual cells but could convey intercellular information among astrocytes [13,14].

The idea that astrocytes are affected by HIV proteins is not new. Previous studies, however, suggested these cells to be not the targets of viral proteins but to participate indirectly in the sequence of events initiated by these proteins elsewhere in the brain [15,16]. In other reports the binding of gp120 to glioma cells and astrocytes or the generation of some signals were reported, however their kinetics was slow and they required gp120 concentrations in the 5×10^{-9}–10^{-7} range [17–21]. Our present observations demonstrate, in contrast, the rapid appearance of classical transduction signals: $[Ca^{2+}]_i$ rises and protein phosphorylation, in a considerable fraction of the rat brain cortex astrocytes when treated with subnanomolar concentrations of gp120. We conclude therefore that astrocytes are very sensitive to a most likely direct action of the viral protein. In HIV-infected individuals the effects induced by gp120 may thus have ample opportunity to affect the array of events mediated by astrocytes in the brain microenvironment [22].

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References

[1] Epstein, L.G. and Gendelman, H.E. (1993) Ann. Neurol. 33, 429–436.
[2] Lipton, S.A. (1992) NeuroReport 3, 913–915.
[3] Ciardo, A. and Meldolesi, J. (1993) Eur. J. Neurosci. 5, 1711–1718.
[4] Codazzi, F., Menegon, A., Zacchetti, D., Ciardo, A., Grohovaz, F. and Meldolesi, J. (1995) Eur. J. Neurosci. 7, 1333–1341.
[5] Lipton, S.A., Sucher, N.J., Kaiser, P.K. and Dreyer, E.B. (1991) Neuron 7, 111–118.
[6] Lipton, S.A. (1994) Nature 367, 113–114.
[7] Blumberg, B.M., Gelbard, H.A. and Epstein, L.G. (1994) Virus Res. 32, 253–267.
[8] McCarthy, K.D. and De Vellis, J. (1980) J. Cell Biol. 85, 890–902.
[9] Meldolesi, J. (1982) J. Neurochem. 38, 1559–1569.
[10] Pozzan, T., Rizzuto, R., Volpe, P. and Meldolesi, J. (1994) Physiol. Rev. 74, 595–637.
[11] Siciliano, J.C., Menegoz, M., Chamak, B. and Girault, J.-A. (1992) Neuroprotocols 1, 185–192.
[12] Matsuda, T., Takuma, K., Nishiguchi, E., Asano, S., Hashimoto, H., Azuma, J. and Baba, A. (1996) Eur. J. Neurosci, in press.
[13] Cornell-Bell, A.H., Finkbeiner, S.M., Cooper, M.S. and Smith, S.J. (1990) Science 247, 470–473.
[14] Charles, A.C., Merrill, J.E., Dirksen, E.R. and Sanderson, M.J. (1991) Neuron 6, 983–992.
[15] Ushijima, H., Nishio, O., Klöcking, R., Perovic, S. and Müller, W.E.G. (1995) Eur. J. Neurosci. 7, 1353–1359.
[16] Dreyer, E.B. and Lipton, S.A. (1995) Eur. J. Neurosci. 7, 2502–2507.
[17] Bath, S., Spitalnik, S.L., Gonsalez-Scarano, F. and Silberberg, D.H. (1991) Proc. Natl. Acad. Sci. USA 88, 7131–7134.
[18] Schneider-Schaulies, J., Schneider-Schaulies, S., Brinkmann, R., Tas, P., Halbrügge, M., Walter, U., Holmes, H.C. and Meulen, V.T. (1992) Virology 191, 765–772.
[19] Benos, D.J., McPherson, S., Hahn, B.H., Chaikin, M.A. and Benveniste, E.N. (1994) J. Biol. Chem. 269, 13811–13816.
[20] Ma, M., Geiger, J.D. and Nath, A. (1994) J. Virol. 68, 6824–6829.
[21] Shrikant, P., Benos, D.J., Tang, L.P. and Benveniste, E.N. (1996) I. Immunol., in press.
[22] Parpura, V., Basarsky, T.A., Liu, F., Jeftinija, K., Jeftinija, S. and Haydon, P.G. (1994) Nature 369, 744–747.