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Binding of HIV-1 gp120 to the nicotinic receptor

Luisa Bracci, Luisa Lozzi, Mauro Rustici and Paolo Neri

Department of Molecular Biology, University of Siena, Policlinico Le Scotte, V. le M. Bracci, 53100 Siena, Italy

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We previously described a significant sequence homology between HIV-1 gp120 and the functional sites responsible for the specific binding of snake curare-mimetic neurotoxins and rabies virus glycoprotein to the nicotinic acetylcholine receptor. Here we report findings about the existence of a mechanism of functional molecular mimicry which could enable the binding of HIV-1 gp120 to nicotinic acetylcholine receptors in muscle cells and neurons.

Acetylcholine receptor; HIV-1; gp120; α-Bungarotoxin; Mimicry

1. INTRODUCTION

In a previous paper [1] we reported a significant homology between the sequence, 164–174 [2], of HIV-1 gp120 and the putative active sites of snake curare-mimetic neurotoxins and rabies virus (RV) glycoprotein, which specifically bind to the nicotinic acetylcholine receptor (AChR). Curare-mimetic neurotoxins from Elapid snakes bind with high affinity to AChR and competitively block acetylcholine-induced membrane depolarization [3]. On the other hand the rabies virus binds to the muscle nicotinic receptor and this binding is inhibited by snake neurotoxins [4]. We consider the homology of gp120 with snake neurotoxins and RV glycoprotein to be of potential importance for HIV-1 infectivity in that it is centered around a region comprising highly conserved snake neurotoxin residues probably involved in receptor binding; moreover, in rabies virus glycoprotein, the same sequence corresponds to the site of rabies virus binding to AChR [5,6].

We proposed that nicotinic acetylcholine receptors can function as HIV-1 receptors in muscle cells and neurones, by virtue of mimicry of receptor-specific active sites of ligands by HIV-1 gp120. A similar mechanism is already suspected for rabies virus binding to muscle cells [6].

We found that recombinant gp120 from HIV-1 strain IIIB is able to inhibit the binding of the snake neurotoxin, α-bungarotoxin (α-Bgt), to the nicotinic acetylcholine receptor in the human rhabdomyosarcoma cell line, TE671. A 14-amino acid synthetic peptide (HG165-178: Asn-Ile-Ser-Thr-Ser-Ile-Arg-Gly-Lys-Val-Gln-Lys-Glu-Tyr), reproducing the sequence 165–178 of gp120, homologous to snake neurotoxins and rabies virus glycoprotein, is also able, once conjugated to keyhole limpet hemocyanin (KLH), to inhibit the binding of α-Bgt to TE671 nicotinic acetylcholine receptor. Further, immunization of mice with the same gp120-derived peptide gave rise to antibodies efficiently cross-reacting with rabies virus glycoprotein and α-Bgt.

Correspondence address: L. Bracci, Department of Molecular Biology, University of Siena, Policlinico Le Scotte, V. le M. Bracci, 53100 Siena, Italy. Fax: (39) (577) 263 302.

2. MATERIALS AND METHODS

2.1. *Cell culture*

The human cell line, TE671, was obtained from the American Type Culture Collection. Cells were grown to confluence at 37°C in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum.

2.2. *Iodination of α-bungarotoxin*

α-Bgt was obtained from Sigma (St. Louis, MO, USA) and labelled with ^{125}I as described [7]; specific activity was 2–3 × 10^{17} cpm/mol.

2.3. *[^{125}I]α-Bgt binding to TE671 cells and inhibition by HIV-1 gp120*

For binding experiments, cells were harvested mechanically with a rubber policeman and centrifuged at 450 × *g* for 15 min; pellets were resuspended in phosphate buffered saline (PBS), pH 7.5, to a density of 10^7 cells/ml; 10^6 cells were incubated with 50 μl of serial dilutions of HIV-1 gp120 (IIIB strain, Neosystem Laboratoire, Strasbourg, France) for 3 h under gentle stirring, 50 μl of [^{125}I]α-Bgt (10^5 cpm) were then added and the cells incubated for a further 45 min. Binding was stopped by the addition of 1 ml ice-cold PBS containing 1 mg/ml BSA and samples were then centrifuged at 450 × *g* for 15 min at 4°C. The cell pellets were washed twice as above and counted in a γ-counter (Minimaxi 500, Packard Instruments Co., Downers Grove, IL). Maximum binding was obtained by replacing inhibitors with assay buffer. Non-specific binding was determined in the presence of 7.5 × 10^{-6} M unlabelled α-Bgt. [^{125}I]α-Bgt binding in the presence of 1 mM nicotine and of 25 mM acetylcholine/0.25 mM neostigmine was also measured for additional controls.

2.4. *Peptide synthesis*

Solid phase synthesis was carried out with a model 430A automatic synthesizer (Applied Biosystems, Foster City, CA) employing F-moc chemistry. The peptide sequence was checked by a gas-phase microse-

quencer (Model 470A, Applied Biosystem). HG165-178 was conjugated with KLH by glutaraldehyde, about 300 mol of peptide were bound per mol of KLH. The conjugated peptide (cHG) was used for inhibition experiments and for immunization of mice.

2.5. *Inhibition of [^{125}I]α-Bgt binding to TE671 AChR by HG165-178 KLH conjugated peptide*

5×10^5 cells in 100 μl of PBS were incubated with 50 μl of serial dilutions of peptides for 2 h. [^{125}I]α-Bgt (10^5 cpm) were then added and incubated for 45 min. The cells were washed and radioactivity counted as described above. Maximum binding was determined by replacing inhibitors with assay buffer. KLH and an uncorrelated 14-amino acid peptide conjugated to KLH (S1) were used under the same conditions to check non-specific inhibition.

2.6. *Antibodies*

Balb/c mice were injected intraperitoneally with 250 μg of HG165-178 KLH-conjugated peptide in complete Freund's adjuvant (CFA) (day 1). The mice were boosted as above using incomplete Freund's adjuvant at days 15 and 36. At day 57, the mice were injected intravenously with 100 μg conjugate in saline. After 3 days serum from immunized animals was collected for the experiments (polyclonal antibodies).

2.7. *ELISA*

Anti-HG165-178 mouse antiserum was tested on three different antigens. 96-well EIA plates were coated with HIV-1 gp120, RV glycoprotein or α-Bgt in 50 mM ammonium carbonate buffer, pH 9.5, for 18 h at 4°C. The plates were then washed and quenched with 3% bovine serum albumin, washed again and incubated with serial dilutions of antiserum for 3 h at 37°C. Binding was detected by horseradish peroxidase-conjugated anti-mouse IgG.

3. RESULTS AND DISCUSSION

A significant homology is present between the sequence, 164–174, of HIV-1 gp120 and the active sites responsible for the binding of snake neurotoxins and rabies virus glycoprotein to muscle AChR (Fig. 1).

The human rhabdomyosarcoma cell line, TE671, is known to express a muscle-like nicotinic receptor [8]. [^{125}I]α-Bgt binding to the nicotinic receptor in the TE671 cell line was measured on intact cells. Non-specific binding was checked in the presence of a high excess of unlabelled α-Bgt. No binding of [^{125}I]α-Bgt was detected on hepatoma PLC/PRF/5 (data not shown), a different human cell line. Gp120 from the HIV-1 strain IIIB was found to inhibit the binding of α-Bgt to TE671: 30% of maximum binding (B_0) was measured in the presence of 2×10^{-7} M gp120 (Fig. 2). Acetylcholine and nicotine were used under the same conditions to specifically inhibit the binding, as already described for α-Bgt binding to torpedo AChR [7,9]. HIV-1 gp120 inhibition of α-Bgt binding indicates a 'functional equivalence' between these proteins for nicotinic receptor binding. To investigate whether the sequence of HIV-1 gp120, homologous to snake neurotoxins and rabies virus glycoprotein, might be involved in the binding of gp120 to AChR, we synthesized a 14-amino acid peptide (HG165-78: Asn-Ile-Ser-Thr-Ser-Ile-Arg-Gly-Lys-Val-Gln-Lys-Glu-Tyr), reproducing the sequence 165–178 of gp120. Once conjugated to KLH this peptide inhibits [^{125}I]α-Bgt binding to intact TE671 cells (Fig. 3). About 40% of maximum binding was obtained in the presence of 1.4×10^{-7} M conjugated peptide, but we could not measure any significant inhibition with the free peptide.

The effect of the protein carrier is not surprising. It has been reported in other cases and attributed to the stabilization of the peptide active conformation [10]. In our case the further ability of gp120 to inhibit the binding of α-Bgt to the nicotinic receptor in TE671 seems to indicate that the HG165-178 active conformation is similar to the one this sequence assumes in the native protein. Moreover immunization of mice with KLH-conjugated HG165-178 gave rise to an antiserum which bound to gp120 and also recognized rabies virus glycoprotein and α-Bgt in ELISA (Fig. 4), confirming

C-D-I-F-T-N-S-R-G-K-R	RV glycoprotein (residues 189-199)
F-N-I-S-T-S-I-R-G-K-V	HIV-1 gp120 (residues 164-174)
C-D-A-F-C-S-I-R-G-K-R	α-cobratoxin (residues 30-40)
C-D-A-F-C-S-S-R-G-K-V	α-bungarotoxin (residues 30-40)

Fig. 1. Sequence homology of HIV-1 gp120 with rabies virus glycoprotein and snake venom neurotoxins.

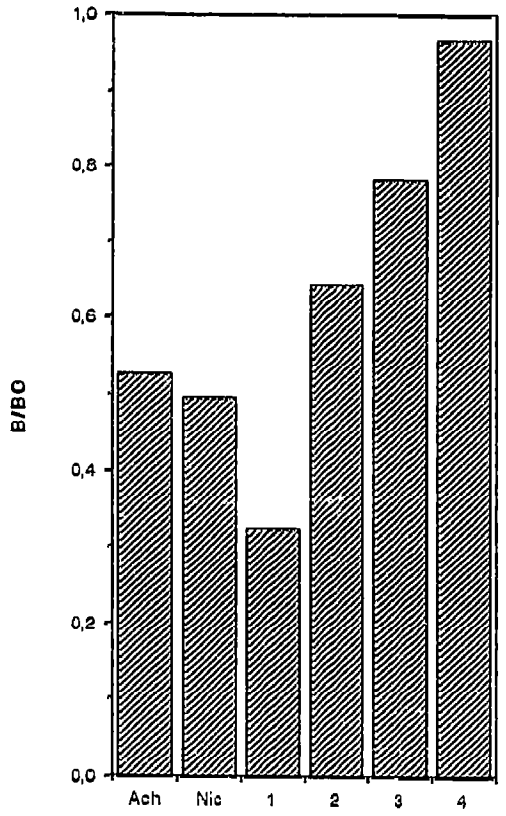

Fig. 2. [^{125}I]α-Bgt binding to TE671 AChR in the presence of (ACh) 2.5×10^{-2} M acetylcholine/2.5×10^{-4} M neostigmine; (Nic) 10^{-3} M nicotine; (1) 2×10^{-7} M gp120; (2) 10^{-7} M gp120; (3) 5×10^{-8} M gp120; (4) 2.5×10^{-8} M gp120. Each point is the mean of duplicate determinations after subtraction of non-specifically bound radioactivity (B), (B_0). Maximum binding (see text).

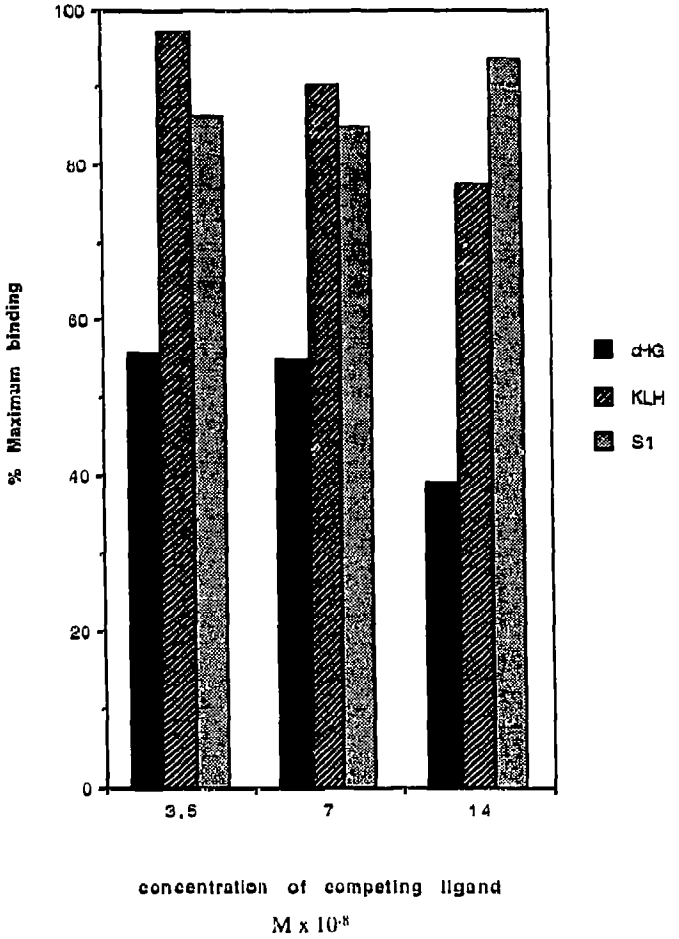

Fig. 3. [^{125}I]α-Bgt binding to TE671 AChR in the presence of different concentrations of the following competing ligands: KLH-conjugated HG165-178 (cHG), KLH, and an uncorrelated 14-amino acid KLH-conjugated peptide (S1).

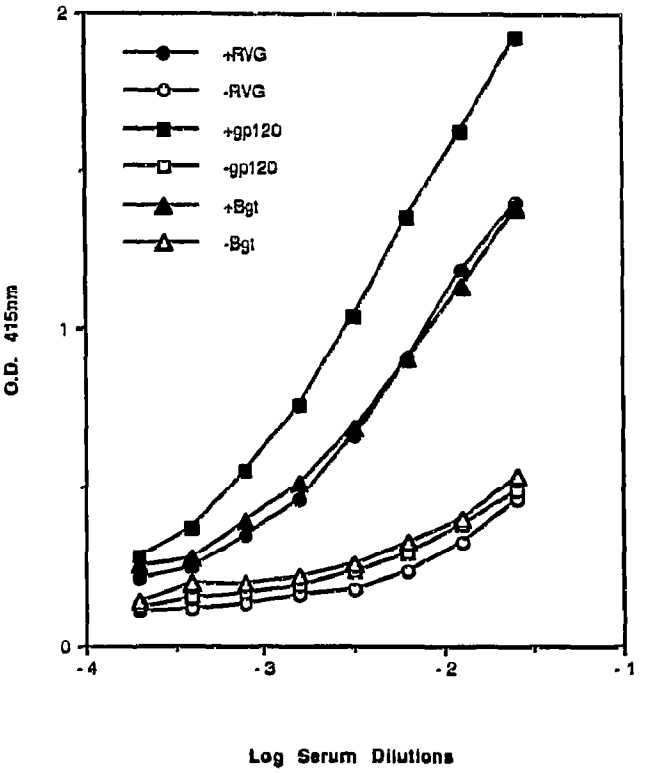

Fig. 4. Anti-KLH-conjugated HG165-178 mouse polyclonal antibodies tested in ELISA on RVG (+RVG), gp120 (+gp120) and α-Bgt (+Bgt), compared to equivalent dilution of normal mouse serum (−RVG), (−gp120) and (−Bgt).

the possibility of a structural similarity between the regions of these proteins having remarkable sequence homology. In the light of our results we cannot exclude the possibility that inhibition of α-Bgt binding to TE671 cells by HIV-1 gp120 is due, at least in part, to receptor down-regulation following gp120 binding.

The existence of an HIV-1 receptor alternative to CD4 in neurones and muscle cells is strongly suggested by evidence of the ability of HIV-1 to infect CD4-negative muscle and neural cells [11,12] and the lack of inhibition by soluble CD4 of HIV-1 infection of muscle and neuronal cell lines [13]. Galactosyl ceramide has been reported to specifically bind HIV-1 gp120, and has been proposed as an essential component of HIV-1 receptors in neural cell lines and brain cells expressing this or a related lipid [14,15]. Our evidence of the binding of gp120 to TE671 nicotinic receptors helps to explain HIV-1 infection of muscle cell lines, and suggests that nicotinic receptors may also bind HIV-1 gp120 in neural cells. At least two populations of nicotinic receptors are expressed in the nervous system [16], one of which is labelled by α-Bgt. Moreover some of the cell lines, such as RD, TE671 and IMR32, in which a CD4-independent infection by HIV-1 has been proposed, are known to express α-Bgt binding nicotinic receptors [8,17].

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REFERENCES

[1] Neri, P., Bracci, L., Rustici, M. and Santucci, A. (1990) Arch. Virol. 114, 265–269.
[2] Wain Hobson, S., Sonigo, P., Danos, O., Cole, S. and Alizon, M. (1985) Cell 40, 9–17.
[3] Endo, T. and Tamiya, N. (1987) Pharmacol. Ther. 34, 403–451.
[4] Lentz, T.L., Burrage, T.G., Smith, A.L., Crick, J. and Tignor, G.H. (1982) Science 215, 182–184.
[5] Lentz, T.L. (1990) J. Gen. Virol. 71, 751–766.
[6] Bracci, L., Antoni, G., Cusi, M.G., Lozzi, L., Niccolai, N., Petreni, S., Rustici, M., Santucci, A., Soldani, P., Valensin, P.E. and Neri, P. (1988) Mol. Immunol. 25, 881–888.
[7] Lindstom, J., Einarson, B. and Tzartos, S. (1981) Methods Enzymol. 74, 432–460.
[8] Schoepfer, R., Luther, M. and Lindstrom, J. (1988) FEBS Lett. 226, 235–240.
[9] Lentz, T.L. (1991) Biochemistry 30, 10949–10957.

[10] Dyson, H.J., Lerner, R.A. and Wright, P.E. (1988) Annu. Rev. Biophys. Biophys. Chem. 17, 305–324.
[11] Harouse, J.M., Kunsch, C., Hartle, H.T., Laughlin, M.A., Hoxie, J.A., Wigdahl, B. and Gonzales-Scarano, F. (1989) J. Virol. 63, 2527–2533.
[12] Li, X.L., Moudgil, T., Vinters, H.V. and Ho, D.D. (1990) J. Virol. 64, 1383–1387.
[13] Clapham, P.R., Weber, J.N., Whitby, D., McIntosh, K., Dalgleish, A.G., Maddon, P.J., Deen, K.C., Sweet, R.W. and Weiss, R.A. (1989) Nature 33, 368–370.
[14] Harouse, J.M., Bhat, S., Spitalnik, S.L., Laughlin, M., Stefano, K., Silberberg, D.H. and Gonzalez-Scarano, F. (1991) Science 253, 320–323.
[15] Bhat, S., Spitalnik, S.L., Gonzales-Scarano, F. and Silberberg, D.H. (1991) Proc. Natl. Acad. Sci. USA 88, 7131–7134.
[16] Deneris, E.S., Connoly, J., Rogers, S.W. and Duvoisin, R. (1991) Trends Pharmacol. Sci. 12, 34–40.
[17] Clementi, F., Cabrini, D., Gotti, C. and Sher, E. (1986) J. Neurochem. 47, 291–297.