Volume 267, number 2, 231–235 FEBS 08597 July 1990

Acetylcholine receptor gene expression in experimental autoimmune myasthenia gravis

Orna Asher[1], Drorit Neumann[1], Veit Witzemann[2] and Sara Fuchs[1]

[1] *Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel* and [2] *Abteilung Zellphysiologie, Max-Planck-Institut für Medizinische Forschung, Heidelberg, FRG*

Received 14 May 1990

Acetylcholine receptor (AChR) gene expression was analyzed in experimental autoimmune myasthenia gravis (EAMG) in rabbits, rats and mice. An increase in AChR transcripts was demonstrated to be exclusively associated with myasthenic symptoms and with a severe loss in membrane AChR. An increase of α-, β-, ε-, and δ-subunit specific mRNAs (5.2-, 1.6-, 3.2- and 3.7-fold, respectively), which code for the adult type of AChR ($\alpha_2\beta\varepsilon\delta$) was observed in EAMG in rats. The γ-subunit transcript was not detectable in myasthenic or healthy rats. It appears that the regulatory control of AChR gene expression in EAMG is different from that observed upon denervation.

Adult and embryonic acetylcholine receptor; Experimental autoimmune myasthenia gravis; Subunit transcript

1. INTRODUCTION

The nicotinic acetylcholine receptor (AChR) is an oligomeric complex of four types of subunits present in a molar stoichiometry of $\alpha_2\beta\gamma\delta$ [1,2]. A fifth subunit called ϵ-subunit was first discovered in calf muscle [3] and was later shown also to exist in rat [4] and mouse muscle [5]. The AChR polypeptides are under developmental control which leads in mammals to the expression of two types of channels [6,7]. At early developmental stages a low conductance channel comprised of α-, β-, γ- and δ-subunits predominates. The channels of the adult muscle have a higher conductance and contain mainly α-, β-, ϵ- and δ-subunits [6]. Upon denervation of adult muscle a large increase in AChR is observed and the majority of these newly synthesized receptors represents low conductance channels consisting of α-, β-, γ- and δ-subunits [4]. The correlation of functional changes, with changes in the content of γ- and ϵ-subunit specific mRNAs, supports the view that the exchange of a single subunit is responsible for the channel conversion observed during development or upon denervation [4,6,7]. AChR is the major autoantigen in myasthenia gravis (MG) and its animal model experimental autoimmune myasthenia gravis (EAMG). Immune attack of AChR at the neuromuscular junction leads to a decrease in the receptor content and impairs signal transmission from nerve to muscle [8].

In a previous report we demonstrated that there are increased levels of AChR α-subunit mRNA in leg muscles of myasthenic rabbits and rats [9]. In the present study we demonstrate that in addition to the increase in the α-subunit mRNA, there is also an elevation in the mRNA for the β-, ϵ- and δ-subunit in myasthenia rats. This type of increase corresponds to an adult class of receptors. We also demonstrate in this report that the increase in AChR mRNA levels correlates exclusively with disease.

Correspondence address: S. Fuchs, Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel

2. MATERIALS AND METHODS

2.1. *AChR preparations*

AChR was purified from *Torpedo californica* electric organ (Pacific Bio-Marine, Venice, CA) as described [10]. Reduced and carboxymethylated AChR (RCM-AChR) was prepared as previously described [11]. Muscle AChR preparations from the hind legs of rabbits and rats were made essentially as described previously [12] and AChR content was determined by measuring binding of [^{125}I]α-bungarotoxin (α-BTX) [10].

2.2. *Immunological procedures*

Immunization of rabbits and rats and induction of EAMG were performed as previously described [9]. Female C57BL/6, SJL/J and SWR mice (10 weeks old) were injected in their footpads with purified AChR (10 μg per mouse) emulsified with complete Freund's adjuvant (CFA). Mice were boosted twice or three times at monthly intervals with 10 μg AChR until myasthenic symptoms were detected. Myasthenic symptoms in mice and rats included motor impairment, ruffled fur, exaggeratedly humped backs, weight loss and fatigability, which was accentuated by exercise. For all species, animals injected with CFA alone were used as controls. Rabbit, mouse and rat sera were tested for antibody titers by solid-phase radioimmunoassay using purified *Torpedo* AChR as the antigen and ^{125}I-labeled protein A for the detection of antibody binding [13].

2.3. *Northern blot analysis*

Rabbits, mice and rats, either healthy or exhibiting EAMG symptoms, were sacrificed and their leg muscles were frozen in liquid N_2,

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

immediately after dissection, for determination of AChR content and for preparation of RNA. Poly(A)$^+$ RNA preparations and Northern blot analysis were performed as described previously [9]. The AChR-specific hybridization probes employed for the α-, β- and δ-subunits were 2 kb *Eco*RI fragments derived from the respective mouse recombinant DNAs [14–16], and were kindly provided by Dr S. Heinemann. The γ- and ϵ-probes were genomic rat fragments as described in [4]. An α-actin specific probe was a gift from Dr U. Nudel.

3. RESULTS

In our previous [9] report we demonstrated that AChR α-subunit mRNA increases in myasthenic animals. In order to find out whether this increase is directly connected to EAMG, or whether it is merely a result of an immune response to AChR, we have analyzed AChR gene expression in rabbits immunized with AChR or with a denatured preparation of AChR (reduced and carboxymethylated AChR, RCM-AChR [11]). As previously reported [11], RCM-AChR-injected rabbits developed anti-AChR antibody titers (Fig. 1A) but did not exhibit myasthenic symptoms. When EAMG was observed in the AChR-injected rabbits, animals from both groups as well as from a control group of animals immunized with CFA, were sacrified and poly(A)$^+$ RNA was prepared from hind leg muscles. Northern blot analysis of the poly(A)$^+$ RNA preparations (Fig. 2) shows an increase (3.75 ± 0.35-fold, calculated on the basis of densitometric analysis of three different rabbits) in the level of the α-subunit mRNA in AChR-injected, myasthenic rabbits, whereas all rabbits immunized with RCM-AChR did not exhibit any significant increase. It should be noted that in earlier preliminary experiments [9] it seemed that an RCM-AChR-injected rabbit exhibited some increase in AChR transcripts, but this observation was not reproduced. The levels of α-actin mRNA were virtually identical in all animals (Fig. 2), reflecting the selective increase in AChR α-subunit mRNA in myasthenic rabbits.

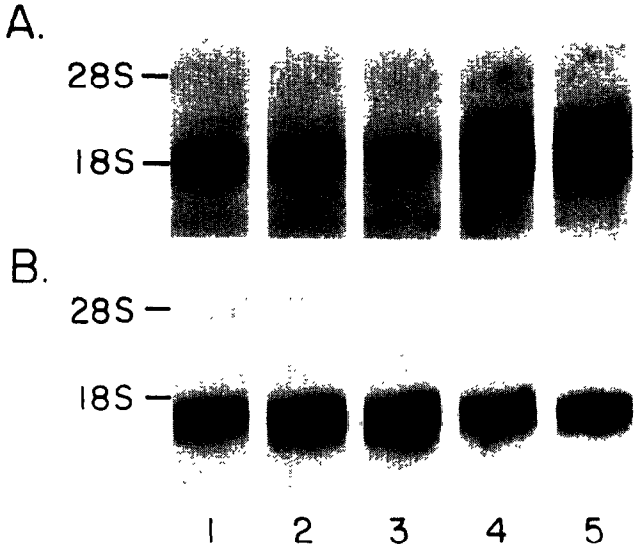

Fig. 2. AChR α-subunit transcripts in rabbits. 10 μg each of poly(A)$^+$ RNA isolated from leg muscle of rabbits injected with CFA (lane 1) RCM-AChR (lanes 2, 3) and AChR (myasthenic) (lanes 4, 5) were size-fractionated on denaturing agarose gel and transferred to Gene Screen plus membrane filters. The blot was then hybridized with an α-subunit cDNA probe from mouse (A) and following stripping, with an α-actin probe (B).

The increase in AChR mRNA levels in myasthenic AChR-injected rabbits was accompanied by 46% decrease in the content of membranal AChR, as compared to its content in control CFA-injected rabbits (31.1 ± 2.4 and 16.7 ± 3.3 fmol α-bungarotoxin (α-BTX) per mg protein in control and myasthenic rabbits, respectively). Some decrease (about 21%) in the surface AChR content was also observed in RCM-AChR-injected, disease-free, rabbits (24.6 ± 4.3 fmol α-BTX/mg protein).

In mice we have analyzed the level of AChR α-subunit mRNA in EAMG susceptible and non-

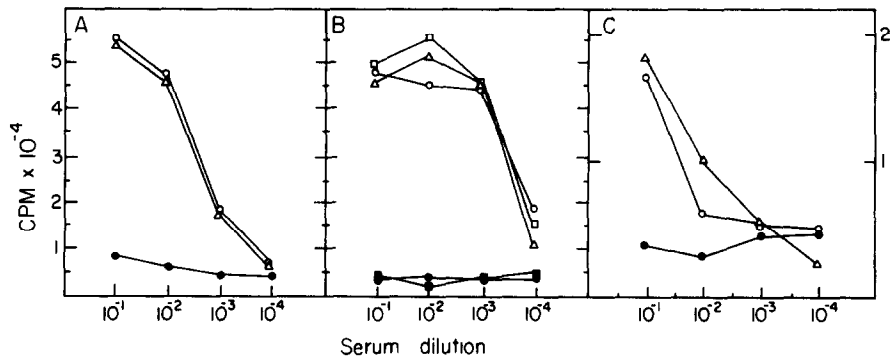

Fig. 1. Anti-AChR antibody titers in rabbits (A), mice (B) and rats (C), as determined by solid phase radioimmunoassay. (A) AChR-injected, myasthenic rabbit (○); RCM-AChR-injected rabbit (△); control, CFA-injected rabbit (●). (B) Myasthenic (○) and nonmyasthenic (△) AChR-injected, and CFA-injected (●) C57BL/6 mice; AChR-injected (□) and CFA-injected (■) SWR mice. (C) Myasthenic (○) and nonmyasthenic (△) AChR-injected rats and CFA-injected (●) rat.

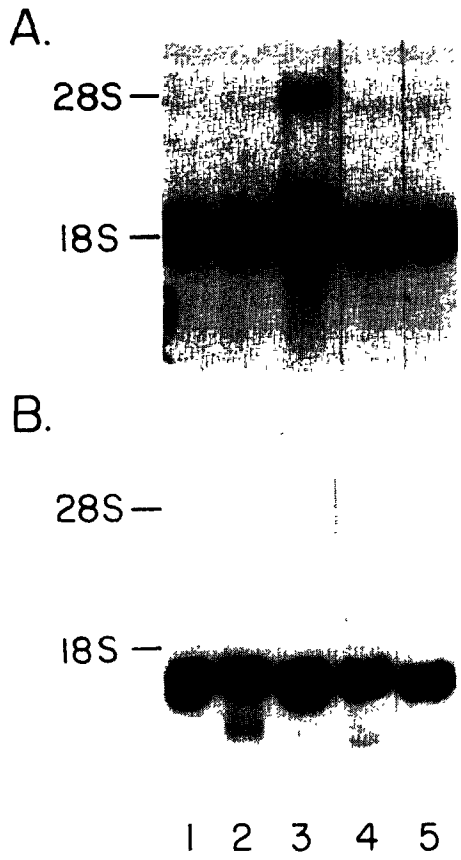

Fig. 3. Northern blot analysis of AChR α-subunit transcripts in mouse muscles, hybridized with AChR α-subunit probe (A) and α-actin probe (B). Poly(A)$^+$ RNA (10 μg) from CFA-injected C57BL/6 (lane 1), AChR-injected (nonmyasthenic) C57BL/6 (lane 2), AChR-injected myasthenic C57BL/6 (lane 3), CFA-injected SWR (lane 4) and AChR-injected (nonmyasthenic) SWR mice (lane 5) were subjected to blot hybridization as described in Fig. 2.

susceptible strains [17]. EAMG susceptible C57BL/6 mice and EAMG nonsusceptible SWR and SJL/J mice, were injected with AChR and examined for antibody titers, for EAMG and for AChR α-subunit mRNA. AChR-immunized mice of all strains developed comparable titers of anti-AChR antibodies (Fig. 1B). Of all AChR-immunized mice, an increase in AChR α-subunit mRNA was observed only in C57BL/6 mice which displayed symptoms of EAMG (Fig. 3). Based on the densitometric evaluation of autoradiograms this increase was 9.1 ± 1.4-fold, as compared to control, CFA-immunized mice, and calculated for three different experiments. AChR-immunized SWR mice (Fig. 3) and SJL/J mice (data not shown) did not display any increase in their AChR α-subunit mRNA. It should be noted that C57BL/6 mice which had been immunized with *Torpedo* AChR, without developing any myasthenic symptoms had no elevated α-subunit mRNA. In accordance with the results in rabbits and mice, increased AChR α-subunit mRNA levels correlated with disease also in rats. Thus, only sick animals exhibited increased levels of AChR α-subunit mRNA, whereas in AChR-immunized rats, which did not show any of the clinical symptoms of EAMG, there was no such increase (Fig. 4). The overall anti-AChR antibody titers of sick and healthy AChR-injected rats were similar (Fig. 1C).

In order to find out whether the observed increase in α-subunit mRNA in myasthenic rats is also seen for the other receptor subunits, the poly(A)$^+$ RNA samples were hybridized with specific probes for the β-, γ-, δ- and ϵ-subunits. As shown in Fig. 4, in addition to the increase in α-subunit mRNA, an increase in the specific transcripts for the β, ϵ and δ was observed. The mRNA for the γ-subunit was undetectable in control as well as in myasthenic rats. The γ-subunit probe employed gave a positive signal when hybridized with RNA from rat primary muscle cell cultures (data not shown). The relative increase in the level of α-, β-, ϵ- and δ-subunit mRNA was calculated to be 5.2-, 1.6-, 3.2- and 3.7-fold, respectively. No significant changes in the level of mRNA transcripts for all AChR subunits were detected in AChR-injected rats which did not exhibit myasthenic symptoms. A decrease in AChR content in myasthenic rats was observed, as has been reported previously [18]. The loss of membranal AChR content was 52% in myasthenic rats and only 27% in AChR-immunized rats which did not display EAMG.

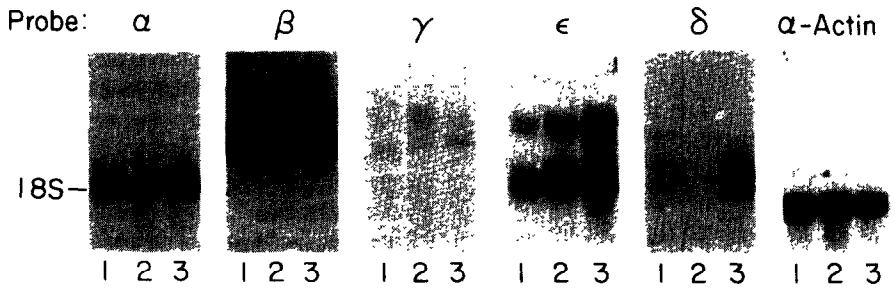

Fig. 4. Northern blot analysis of transcripts of AChR subunits in rat muscles. Poly(A)$^+$ RNA (10 μg) from hind leg muscle were analyzed by blot hybridization using AChR α-, β-, γ-, ϵ- and δ-DNA probes, and α-actin-specific DNA probe. For each probe, RNA from control CFA-injected (lane 1), nonmyasthenic AChR-injected (lane 2) and myasthenic AChR-injected (lane 3) rats was analyzed as described in Fig. 2.

4. DISCUSSION

In this report we have shown that an increase in AChR transcripts correlates exclusively with the expression of EAMG. Only animals which exhibit myasthenic symptoms display increased levels of AChR-mRNA. On the other hand, animals with high anti-AChR antibody titers which do not have myasthenia, such as rabbits immunized with RCM-AChR or AChR-immunized rats and mice that were not sick, did not have increased levels of AChR transcripts. It is possible that only a distinct population of antibodies which damage the neuromuscular junction and interfere with proper muscle activity can result in altered regulation of gene expression.

It has been reported that the levels of surface membrane AChR are reduced in EAMG [18,19]. We have also observed a reduction in membranal AChR content in myasthenic rabbits and rats (46% and 52%, respectively) along with an increase in AChR gene expression. These reduced levels of AChR content may represent an essentially higher loss in AChR, which is partially compensated by elevated amounts of newly synthesized AChR, resulting from the increase in AChR transcripts. It should be noted that some decrease in AChR content was also detected in RCM-AChR-injected rabbits (21%) and in nonmyasthenic AChR-injected rats (27%). These nonmyasthenic animals did not display increased AChR-mRNA levels. Possibly, the increase in AChR transcripts is triggered only above a certain threshold loss in AChR content, which is also accompanied by damage to the neuromuscular junction.

Messenger RNA levels for the AChR subunits were shown to be regulated by denervation, embryonic development and muscle activity. Denervation resulted in an increase of AChR transcripts [20–24]. Specifically, an increase in the levels of mRNA coding for the α-, β-, γ- and δ-subunits of AChR was observed following denervation, whereas only a small increase in the mRNA coding for the ϵ-subunit was observed [4]. Thus, denervation results mainly in an increased level of AChR transcripts corresponding to the embryonic ($\alpha_2\beta\gamma\delta$) receptor, accompanied by the elevation of the respective extrajunctional AChR. In our study on myasthenic rats we observed an increase in the level of the ϵ-subunit mRNA (3.2-fold) whereas the γ-subunit transcripts remained undetectable. The increase in the ϵ-subunit mRNA is relatively similar to the increase of the α-, β- and δ-subunit mRNA (5.2-, 1.6- and 3.7-fold, respectively). In agreement with previous reports on denervation [24] we have also observed that the amount of β-subunit transcript in control animals was high relative to the other subunit transcripts (Fig. 4), and increased less than the α-, ϵ- and δ-transcripts in EAMG.

It appears from our experiments that EAMG is accompanied by a moderate increase in the level of AChR transcripts which corresponds to the adult, junctional receptor ($\alpha_2\beta\epsilon\delta$). It is thus reasonable to anticipate that if more AChR transcripts are being made as a compensatory mechanism, they will be of the type which is being lost and needed, i.e. the adult form. We do not know yet whether the observed increased AChR mRNA levels in EAMG result in an increase in newly synthesized adult AChR, and whether this newly synthesized AChR is properly inserted into the end plate. In this respect it should be mentioned that earlier studies by Cull-Candy et al. [25] demonstrated that the properties of the end plate channels (single channel conductance and mean channel-life time) in myasthenia gravis are identical to the properties of the channel in normal muscle. This may support the notion that the newly made junctional AChR in EAMG is of the adult form (i.e. $\alpha_2\beta\epsilon\delta$).

In conclusion, we have shown that regulation of AChR gene expression is modulated in EAMG and might be important for elucidating the mechanism and control of myasthenia. This regulation seems to be different from that observed in denervation and provides a different physiological condition for resolving the diverse mechanisms controlling muscle AChRs.

Acknowledgements: We thank Professor B. Sakmann for encouragement and fruitful discussions, Professor S. Heinemann for the mouse AChR probes, Professor U. Nudel for the α-actin probe, and D. Barchan for excellent technical assistance. This research was supported by grants from the Los Angeles Chapter of the Myasthenia Gravis Foundation, the Muscular Dystrophy Association of America, the Association Française Contre les Myopathies (AFM), and the United States-Israel Binational Science Foundation (BSF).

REFERENCES

[1] Karlin, A. (1980) in: The Cell Surface and Neuronal Functions (Cotman, C.W., Poste, G. and Nicolson, G.L. eds) pp. 191–250, Elsevier, Amsterdam.
[2] Popot, J.L. and Changeux, J.-P. (1984) Phys. Rev. 64, 1162–1139.
[3] Takai, T., Noda, M., Mishina, M., Shimizu, S., Furutani, Y., Kayano, T., Ikeda, T., Kubo, T., Takahashi, H., Takahashi, T., Kuno, M. and Numa, S. (1985) Nature 315, 761–764.
[4] Witzemann, V., Bary, B., Nishikawa, Y., Sakmann, B. and Numa, S. (1987) FEBS Lett. 223, 104–112.
[5] Buonanno, A., Mudd, J. and Merlie, J.P. (1989) J. Biol. Chem. 264, 7611–7616.
[6] Mishina, M., Takai, T., Imoto, K., Noda, M., Takahashi, T., Numa, S., Methfessel, C. and Sakmann, B. (1986) Nature 321, 406–411.
[7] Witzemann, V., Barg, B., Criado, M., Stein, E. and Sakmann, B. (1989) FEBS Lett. 242, 419–424.
[8] Lindstrom, J., Shelton, D. and Fugii, Y. (1988) Adv. Immunol. 42, 233–284.
[9] Asher, O., Neumann, D. and Fuchs, S. (1988) FEBS Lett. 233, 277–281.
[10] Aharonov, A., Tarrab-Hazdai, R., Silman, I. and Fuchs, S. (1977) Immunochemistry 14, 129–137.
[11] Bartfeld, D. and Fuchs, S. (1977) FEBS Lett. 77, 214–218.
[12] Souroujon, M.C., Mochly-Rosen, D., Gordon, A.S. and Fuchs, S. (1983) Muscle Nerve 6, 303–311.

[13] Mochly-Rosen, D. and Fuchs, S. (1981) Biochemistry 20, 5920–5924.
[14] Boulter, J., Luyten, W., Evans, K., Mason, P., Ballivet, M., Goldman, D., Stengelin, S., Martin, G., Heinemann, S. and Patrick, J. (1985) Proc. Natl. Acad. Sci. USA 5, 2545–2552.
[15] Lapolla, R.J., Mayne, K.M. and Davidson, N. (1984) Proc. Natl. Acad. Sci. USA 81, 7970–7974.
[16] Buonanno, A., Mudd, J., Shah, O. and Merlie, J.P. (1986) J. Biol. Chem. 261, 16451–16458.
[17] Fuchs, S., Nevo, D., Tarrab-Hazdai, R. and Yaar, I. (1976) Nature 263, 329–330.
[18] Lindstrom, J.M., Einarson, B.L., Lennon, V.A. and Seybold, M.E. (1976) J. Exp. Med. 144, 726–738.
[19] Drachman, D.B., Kao, I., Pestronk, A. and Toyka, K.F. (1976) Ann. NY Acad. Sci. 274, 226–234.
[20] Merlie, J.P., Isenberg, K.E., Russel, S.D. and Sanes, J.R. (1984) J. Cell Biol. 99, 332–335.
[21] Klarsfeld, A. and Changeux, J.-P. (1985) Proc. Natl. Acad. Sci. USA 82, 4558–4562.
[22] Goldman, D., Boulter, J., Heinemann, S. and Patrick, J. (1985) J. Neurosci. 5, 2553–2558.
[23] Goldman, D., Brenner, H.R. and Heinemann, S. (1988) Neuron 1, 329–333.
[24] Evans, S., Goldman, D., Heinemann, S. and Patrick, J. (1987) J. Biol. Chem. 262, 4911–4916.
[25] Cull-Candy, S.G., Miledi, R. and Trautmann, A. (1978) Nature 271, 74–75.