Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 1983–1987, March 1988
Neurobiology

Acetylcholine receptor-inducing factor from chicken brain increases the level of mRNA encoding the receptor α subunit

(neuromuscular/neurotrophic/synaptogenesis/myotube/nuclease protection)

DAVID A. HARRIS, DOUGLAS L. FALLS, REBECCA M. DILL-DEVOR, AND GERALD D. FISCHBACH

Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110

Contributed by Gerald D. Fischbach, November 10, 1987

ABSTRACT **A 42-kDa glycoprotein isolated from chicken brain, referred to as acetylcholine receptor-inducing activity (ARIA), that stimulates the rate of incorporation of acetylcholine receptors into the surface of chicken myotubes may play a role in the nerve-induced accumulation of receptors at developing neuromuscular synapses. Using nuclease-protection assays, we have found that ARIA causes a 2- to 16-fold increase in the level of mRNA encoding the α subunit of the receptor, with little or no change in the levels of γ- and δ-subunit messengers. ARIA also increases the amount of a putative nuclear precursor of α-subunit mRNA, consistent with an activation of gene transcription. These results suggest that the concentration of α subunit may limit the rate of biosynthesis of the acetylcholine receptors in chicken myotubes. They also indicate that neuronal factors can regulate the expression of receptor subunit genes in a selective manner. Tetrodotoxin, 8-bromo-cAMP, and forskolin also increase the amount of α-subunit mRNA, with little change in the amount of γ- and δ-subunit mRNAs. Unlike ARIA, however, these agents have little effect on the concentration of the α-subunit nuclear precursor.**

Motor neurons induce the accumulation of acetylcholine receptors (AcChoRs) at developing neuromuscular junctions (reviewed in refs. 1–3). Two mechanisms have been found to contribute to this phenomenon. Motor neurons may promote the aggregation of receptors that were present on the myocyte surface before nerve–muscle contact, and they may increase the insertion of new receptors in the immediate vicinity of the synapse (4). Receptor aggregation is evident at chicken junctions, but, during the first 24 hr after contact, newly inserted receptors make up the great majority of those present (7). Several laboratories have characterized factors from neural tissue that alter the number or distribution of AcChoRs on cultured myotubes and that may, therefore, play a role in the accumulation of receptors at nascent synapses (8–14). Little is known, however, about the molecular mechanisms by which these factors act.

We have purified a 42-kDa glycoprotein from chicken brain, called ARIA (for *A*cCho*R*-*i*nducing *a*ctivity), that stimulates the rate of insertion of AcChoRs into the membrane of chicken myotubes by as much as 5-fold at picomolar concentrations (15). We were interested in determining which steps of receptor biosynthesis were altered by this factor. Synthesis of the AcChoR is a complex process consisting of transcription of the genes encoding each of the four receptor subunits, translation of the corresponding mRNAs, covalent and conformational modification of the polypeptide chains, assembly of the subunits into a pentameric complex, and transport to the cell surface (reviewed in ref. 16). We report here that the 42-kDa ARIA increases the level of mRNA encoding the α but not the γ or δ subunits of the AcChoR.

MATERIALS AND METHODS

Cell Culture. Mononucleated cells were dissociated from pectoral muscles of 11- to 12-day-old chicken embryos and seeded on 35-mm collagen-coated culture dishes (see ref. 11). In order to obtain sufficient RNA for analysis, cells were plated at high density (1.5×10^6 per dish) and were not treated with 1-β-D-arabinofuranosyl cytosine (cytosine arabinoside).

Purification of ARIA. ARIA was extracted from chicken brains in a trifluoroacetic acid-containing cocktail and purified by high-pressure liquid chromatography on reverse-phase and ion-exchange supports (15). The preparation used in our experiments consisted of the most active fraction from the fourth chromatographic step, a Vydac C_{18} column eluted with an acetonitrile gradient in heptafluorobutyric acid (F_7BtOH).

The F_7BtOH fraction contained 17 μg of protein per ml (determined by amino acid analysis) and produced a half-maximal effect in our standard assay at a concentration of 10 ng/ml (specific activity = 99,000 units/mg; ref. 15). Since completely purified 42-kDa ARIA has a specific activity of 1.7×10^6 units/mg, we estimate that the 42-kDa polypeptide represented approximately 6% of the protein in the sample we used. This constitutes a purification of 40,000-fold compared to a saline extract of chicken brain.

Assay of AcChoR Insertion Rate. The rate of insertion of new AcChoRs into the myotube surface was assayed as described previously (15, 17). Briefly, all surface receptors were blocked with unlabeled α-bungarotoxin (α-BTX; 100 nM, 60 min, 37°C), and the number of new receptors inserted after 1 or 5 hr was determined by binding of ^{125}I-labeled α-BTX (5 nM, 60 min, 37°C).

Preparation of RNA. Total RNA was extracted from the same cultures used for the ^{125}I-labeled α-BTX assay by solubilization in guanidine monothiocyanate and precipitation with LiCl (18). Nuclear and cytoplasmic RNA were prepared as described by Maniatis *et al.* (19).

In one experiment, the average amount of RNA per myotube nucleus was calculated by dividing the yield of RNA per plate by the number of muscle nuclei per plate. RNA yields were corrected for loss by measuring the recovery of a ^{32}P-labeled RNA transcript (see Fig. 1*D*) that was added to muscle samples after the samples were solubilized in guanidine monothiocyanate.

Hybridization Probes. ^{32}P-labeled antisense RNA probes were synthesized with SP6 or T7 polymerase by the procedure of Melton *et al.* (20) using the templates shown in Fig. 1 *A–D*. Templates were constructed by subcloning into the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: AcChoR, acetylcholine receptor; ARIA, AcChoR-inducing activity; α-BTX, α-bungarotoxin; F_7BtOH, heptafluorobutyric acid; TTX, tetrodotoxin; nt, nucleotide(s).

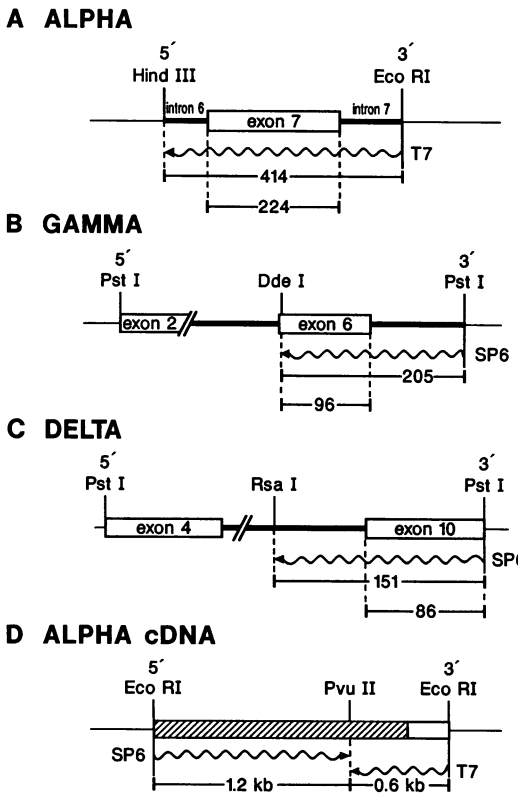

FIG. 1. Genomic and cDNA templates and the RNA probes synthesized from them. The templates were constructed by subcloning the following restriction fragments into the transcriptional vector pGEM-1: *(A)* the *Hin*dIII–*Eco*RI fragment from pX2, a genomic clone of the chicken α subunit; *(B)* the *Pst* I fragment from p9, a genomic clone of the chicken γ subunit; *(C)* the *Pst* I fragment from p1.6, a genomic clone of the chicken δ subunit; and *(D)* the *Eco*RI fragment from p114L7, a cDNA clone of the chicken muscle α subunit. Exons are indicated by open boxes, introns by heavy lines, and vector sequence by light lines. The hatched region in *D* indicates the coding region of the messenger. Each template was linearized at the indicated restriction site, and an RNA probe (wavy line) was synthesized from either the T7 or SP6 promoter. The sizes (in nt) of each probe and of the exon protected by the complementary mRNA are indicated. Probe sizes include the small amount of vector sequence that lies between the promoter and the first nucleotide of the insert. In *A*, intron 6 is 70 nt and intron 7 is 105 nt (21). The antisense probes shown in *A–C* were used in nuclease protection assays and blot hybridizations. The 0.6-kilobase antisense RNA shown in *D* was used to probe RNA blots, and the 1.2-kilobase sense-strand RNA was used to calibrate the nuclease protection assay (see *Materials and Methods*).

transcriptional vector pGEM-1 (Promega Biotec, Madison, WI) the following restriction fragments from plasmids kindly provided by Marc Ballivet (Université de Genève): *Hin*dIII–*Eco*RI fragment from pX2, a genomic clone of the chicken α subunit (22); *Pst* I fragments from p9 and p1.6, genomic clones of the chicken γ and δ subunits (23); *Eco*RI fragment from p114L7, a cDNA clone of the chicken muscle α subunit. The specific activity of each probe was 2.8×10^8 dpm/μg, except in some experiments where it was 8.4×10^8 dpm/μg.

Nuclease-Protection Assay and RNA Blot Hybridization. Nuclease-protection assays were carried out exactly as described by Melton *et al.* (20). Briefly, 1.5–10 μg of muscle RNA was hybridized in 10 μl at 45°C with 4×10^5 dpm of the labeled probe, digested with RNase A and T1 for 30 min at room temperature, and then analyzed on urea polyacrylamide gels.

Autoradiographic signals were quantitated by densitometric scanning at 550 nm in a Beckman DU-30 spectrophotometer. The amount of radioactivity in the nuclease-resistant fragment was determined by comparison to the autoradiographic signal produced by a known amount of undigested probe run on the same gel. The specific activity of the probe was then used to calculate the number of attomoles of mRNA. Signals for α-subunit mRNA were also compared to a set of standards, which consisted of known amounts of sense-strand RNA (Fig. 1*D*) assayed in parallel. The two methods of calculation agreed to within 20%.

RNA was electrophoresed in formaldehyde-containing agarose gels, blotted onto GeneScreen (New England Nuclear), and hybridized, all by standard procedures (19).

RESULTS

AcChoR mRNA in Control Myotubes. The α-, γ-, and δ-subunit mRNAs were quantitated by using a sensitive RNase-protection assay in which exon-containing regions of a ^{32}P-labeled probe base-pair with complementary sequences in the mRNA and are thereby protected from attack by nucleases specific for single-stranded RNA. No probe for the β subunit of the receptor was available.

RNA from muscle cultures protected a segment of the α-subunit probe that was 224 nucleotides (nt) long (Fig. 2), corresponding to exon 7 of the gene (see Fig. 1*A*). A 414-nt RNA that was observed inconsistently most likely represents a small amount of undigested probe. A fragment of approximately 285 nt was detected in each assay and was 16 ± 3% (mean ± SEM; n = 9 platings) of the amount of the 224-nt fragment. This larger fragment was absent when the probe was hybridized with bacterial tRNA, and hence it is probably not an artifact resulting from secondary structure

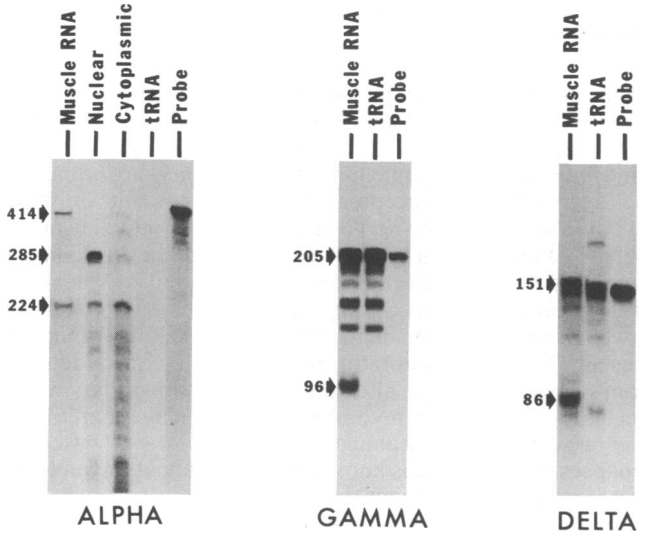

FIG. 2. Nuclease-protection assay of AcChoR mRNA from control myotubes. Probes for receptor subunits were hybridized with 10 μg of total RNA from control myotubes (lanes labeled "muscle RNA"), 10 μg of *Escherichia coli* tRNA, and, for the α-subunit probe, 25 μg of nuclear or cytoplasmic RNA from control myotubes. Following digestion with RNases A and T1, samples were electrophoresed in a polyacrylamide gel (5% for α, 8% for γ and δ) containing 8 M urea and subjected to autoradiography. The lanes labeled "probe" contain 1–1.5 pg of the corresponding probe that was not digested with RNase. The positions of the undigested probe, the protected exon, and a 285-nt nuclear precursor (for α) are indicated. Sizes were determined by comparison to *Msp* I-digested pBR322 markers run on the same gel. Autoradiographic exposures were 29 hr for α (except nuclear and cytoplasmic RNA), 16 hr for γ, and 52 hr for δ. Nuclear and cytoplasmic RNA were analyzed in a separate experiment and were exposed for 16 hr and 5 days, respectively.

of the probe. The 285-nt fragment must contain intron sequences—most likely the 70 nt of intron 6 present in the probe—as well as all of exon 7 (total of 294 nucleotides; see Fig. 1*A*). This fragment is probably derived from a partially spliced precursor of the mature α-subunit mRNA. In support of this idea, the 285-nt fragment is enriched in nuclear RNA preparations (Fig. 2 *Left*). Using the same probe, Shieh *et al.* (21) observed 224- and 294-nt-protected fragments when RNA from chicken limb muscle was analyzed, although the proportion of the larger fragment was only about 2% of the total. They also detected a third fragment corresponding to exon 7 plus introns 6 and 7 (total of 399 nt), but we did not resolve this species, which would migrate close to the undigested probe.

The γ- and δ-subunit probes produced nuclease-resistant fragments of 96 and 86 nt, respectively (Fig. 2), which correspond to the single exon contained in each probe (see Fig. 1 *B* and *C*). Several additional fragments were observed, but each one was evident when the probes were hybridized with tRNA.

The number of attomoles of the mRNAs per milligram of total RNA in control myotubes was as follows: α, 602 ± 192 (mean ± SEM; n = 10 platings); γ, 2758 ± 600 (n = 5); δ, 481 ± 41 (n = 5). Although the amount of mRNA for the α subunit was similar from dish to dish in a given plating, different platings exhibited a 10-fold variation. In contrast, the concentrations of mRNA for the γ and δ subunits were more constant between platings. The amount of RNA per myotube nucleus was 107 ± 2.8 pg (mean ± SEM; n = 3 plates), which implies that the average number of copies of the three mRNAs per myotube nucleus was about 40 for the α and δ subunits and 200 for the γ subunit. *In situ* hybridization experiments indicate, however, that some nuclei are associated with more α-subunit mRNA than others (D.A.H. and G.D.F., unpublished work).

ARIA Increases α-Subunit mRNA. When applied to chicken myotubes for 24 hr, ARIA increased the amount of the 224-nt fragment; the effect varied between 2-fold and 16-fold (Fig. 3 and Table 1). The level of the 285-nt fragment was also increased, but to a lesser extent. There was an inverse relation between the control level of α-subunit mRNA and the fold increase produced by ARIA, which suggests the existence of a maximal rate of stimulation under our conditions.

In contrast, ARIA produced little alteration in the concentrations of γ- and δ-subunit mRNAs. In one experiment, these mRNAs increased in amount by only 20%, even though α-subunit mRNA rose 4.8-fold, and in a second experiment they remained unchanged, although α-mRNA increased by 16.5-fold.

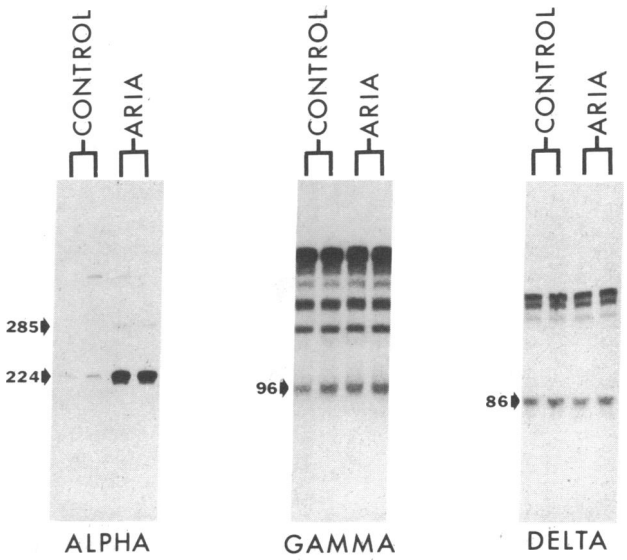

FIG. 3. Effect of ARIA on the amounts of AcChoR subunit mRNAs. Total RNA (5 μg for α and γ; 10 μg for δ) from duplicate control and ARIA-treated cultures was hybridized and digested with RNase. The ARIA-containing F_7BtOH fraction was added to myotubes for 24 hr starting on the sixth day after plating at a final concentration of 340 ng/ml. The position of the protected exon is shown for each subunit, as well as the location of the putative nuclear precursor of α-subunit mRNA (285 nt). Autoradiographic exposures were 18.5 hr (α), 16 hr (γ), or 52 hr (δ). The experiment shown here is listed as experiment 1 in Table 1.

The effect on α-subunit mRNA depended on the concentration of ARIA; a clear increase was observed with as little as 4.5 ng/ml of the F_7BtOH fraction (not shown). Since this preparation is estimated to be 6% pure (see *Materials and Methods*), this concentration is equivalent to 6 pM 42-kDa ARIA.

In four experiments we measured the rate of incorporation of new AcChoRs into the myotube surface and the level of subunit mRNAs in the same cultures (Table 1). In two cases (experiments 3 and 6), the fold increase in α-subunit mRNA produced by ARIA was similar to the fold increase in receptor incorporation rate, but in two other cases (experiments 1 and 4), the change in messenger level greatly exceeded the stimulation of the incorporation rate. In no case did the mRNA level increase less than the rate of AcChoR incorporation.

In two experiments where the α-subunit mRNA level and the AcChoR insertion rate were measured as a function of time in the same cultures, the increase in mRNA level preceded the rise in receptor insertion (data not shown). We

Table 1. Effect of ARIA on AcChoR mRNA levels and incorporation rate

	α-Subunit mRNA, amol/mg of RNA				γ-Subunit mRNA, amol/mg of RNA		δ-Subunit mRNA, amol/mg of RNA		AcChoR incorporation, fmol/hr per plate	
	224-nt fragment		285-nt fragment		96-nt fragment		86-nt fragment			
Exp.	Control	ARIA	Control	ARIA	Control	ARIA	Control	ARIA	Control	ARIA
1	232	3828 (16.5)	26.3	118 (4.5)	1951	2029 (1.0)	489	411 (0.8)	2.54	4.06 (1.6)
2	266	3777 (14.2)	41.4	215 (5.2)						
3	338	1048 (3.1)	116	592 (5.1)					4.07	11.0 (2.7)
4	538	2582 (4.8)	46	143 (3.1)	1631	1941 (1.2)	454	540 (1.2)	2.58	5.16 (2.0)
5	918	3672 (4.0)	113	249 (2.2)						
6	2086	4172 (2.0)	352	563 (1.6)					4.94	6.92 (1.4)

mRNA levels were determined by nuclease protection assays, and the AcChoR incorporation rate was determined by binding of ^{125}I-labeled α-BTX. The fold increase over the control value is given in parentheses. The F_7BtOH fraction was added for 24 hr at the following concentrations: 340 ng/ml on day 6 after plating (experiment 1); 230 ng/ml on day 6 (experiment 2) or day 7 (experiment 5); 570 ng/ml on day 5 (experiment 3); 110 ng/ml on day 6 (experiment 4) or day 5 (experiment 6). Values are the average of duplicate plates, except for experiments 3 and 4 where values for single plates are reported.

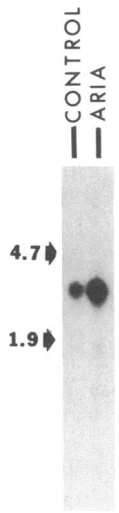

FIG. 4. Blot-hybridization analysis of α-subunit mRNA. Total RNA (10 μg) from control and ARIA-treated cultures was electrophoresed in a 1% agarose/2.2 M formaldehyde gel, transferred to GeneScreen, and hybridized to a 0.6-kilobase antisense RNA probe for the α subunit (see Fig. 1*D*). The F_7BtOH fraction was added to a final concentration of 110 ng/ml for 24 hr starting on the sixth day after plating. Size markers are 18S and 28S rRNA. The same RNA samples were analyzed by nuclease protection in experiment 4 in Table 1.

do not yet know the precise time course of ARIA's action, but in one experiment an elevation of α-subunit mRNA was evident 4 hr after ARIA addition but not at 2 hr; it was maximal at 6 hr.

Gel blots of muscle RNA were hybridized with ^{32}P-labeled antisense RNA derived from either a cDNA or genomic clone of the α-subunit (see Fig. 1 *A* and *D*). A single mRNA of 3.0 kilobases was observed in both control and ARIA-treated myotubes (Fig. 4), suggesting that ARIA does not induce the synthesis of an alternate form of the α subunit. We did not observe a second RNA corresponding to the putative nuclear precursor, perhaps because it is too close in size to the mature α-subunit mRNA to be resolved on our gels or because in the preparation analyzed it was present in amounts too low to be detected.

Gel blots hybridized with antisense RNA synthesized from either γ- or δ-subunit genomic templates (see Fig. 1 *B* and *C*) displayed a broad band centered at 1.9 kilobases (not shown). In some experiments the δ-subunit probe also hybridized to an RNA of 4.7 kilobases.

Tetrodotoxin and Intracellular cAMP Also Increase α-Subunit mRNA. Both tetrodotoxin (TTX) (24) and cAMP (25, 26) increase the number of AcChoRs on cultured myotubes, and TTX elevates the level of α-subunit mRNA detected by RNA blot hybridization (27). We found that TTX, 8-bromo-cAMP, and forskolin all increased the amount of the 224-nt fragment protected by α-subunit mRNA and that like ARIA they had little effect on γ- and δ-subunit mRNAs (Table 2). Unlike ARIA, however, these agents had little effect on the amount of the 285-nt fragment corresponding to the putative nuclear precursor of α-subunit mRNA.

DISCUSSION

We have shown that a glycoprotein purified from chicken brain that increases the rate of AcChoR incorporation into the surface of cultured myotubes also increases the amount of mRNA encoding the α subunit of the receptor. The magnitude and timing of the effect on α-subunit mRNA are sufficient to account for the observed stimulation of AcChoR insertion into the surface membrane. However, our results do not rule out an additional effect of ARIA on other steps in the biosynthesis of the receptor, such as the efficiency with which receptor subunits are assembled or transported to the cell surface (see refs. 28 and 29).

Our results suggest that ARIA stimulates transcription of the α-subunit gene, since it increases the amount of a 285-nt-protected fragment, which is likely to be derived from a nuclear precursor of α-subunit mRNA. Transcriptional activation may not account completely for the action of ARIA on α-subunit mRNA, however, since in some experiments the increase in the amount of the 285-nt fragment was considerably less than that of the 224-nt fragment. Direct measurement of transcription and degradation rates will help resolve this issue.

The striking effect of ARIA on α-subunit mRNA occurred with little or no alteration in the amounts of mRNA encoding the γ and δ subunits. The simplest interpretation of these results is that the amount of α subunit limits biosynthesis of the AcChoR in uninnervated chicken myotubes. In fact, we find that the molar ratio of α to γ to δ subunit mRNAs in untreated chicken myotubes averages 1:4.6:0.8. (No probe for the β subunit was available.) If each of the mRNAs were translated with equal efficiency, then α subunits would be present in limiting amounts for assembly of a receptor with the stoichiometry $\alpha_2\beta\gamma\delta$. Other subunits might become limiting once the concentration of α subunits exceeded a certain level. This might have occurred in experiment 1 of Table 1, where ARIA produced a 16.5-fold increase in α-subunit mRNA but only a 1.6-fold increase in the rate of AcChoR insertion.

Our results indicate that expression of receptor subunit genes can be regulated independently of each other. Consistent with this notion, expression of the bovine ε-subunit gene is selectively activated postnatally, a process that probably depends on the presence of the motor neuron (30). It is possible that trophic agents in addition to ARIA selectively alter the amounts of β-, γ-, and δ-subunit mRNAs

Table 2. Comparison of ARIA with other agents that increase the AcChoR incorporation rate

Agent	α-Subunit mRNA		γ-Subunit mRNA	δ-Subunit mRNA	AcChoR incorporation
	224-nt fragment	285-nt fragment	96-nt fragment	86-nt fragment	
ARIA	7.4 ± 2.5 (6)	3.6 ± 0.6 (6)	1.1 ± 0.1 (2)	1.0 ± 0.2 (2)	1.9 ± 0.3 (4)
TTX	5.6 ± 0.6 (5)	1.3 ± 0.3 (3)	1.1 ± 0.1 (3)	1.5 ± 0.1 (3)	4.8 ± 1.4 (5)
8-Bromo-cAMP	3.7 ± 0.7 (2)	0.8 ± 0.1 (2)	1.0 ± 0.0 (2)	0.9 ± 0.0 (2)	1.5 (1)
Forskolin	5.9 ± 0.7 (2)	1.4 ± 1.0 (2)	1.1 ± 0.1 (2)	1.0 ± 0.1 (2)	2.4 (1)

The average values of duplicate control and ARIA-treated plates were used to calculate the fold increase (over the control) in all but two of the ARIA experiments and two of the TTX experiments, where values for single plates were used. The values reported are the means ± SEM for the number of experiments given in parentheses. The ARIA experiments are those listed in Table 1. TTX was added at a final concentration of 1 μM from day 4 to 6 (two experiments), day 6 to 7 (two experiments), or day 6 to 8 (one experiment) after plating. 8-Bromo-cAMP was added at a concentration of 1 mM from day 6 to 7; in half of the plates 0.1 mM 3-isobutyl-1-methylxanthine was also present. Forskolin was added at a concentration of 0.1 mM from day 6 to 7.

and that these factors act in concert to produce a maximal stimulation of receptor synthesis during synapse formation.

In other situations where alterations in the amounts of receptor mRNAs have been analyzed, a selective effect on the α-subunit messenger has not been observed. Denervation of mouse leg muscle markedly increases the levels of all four subunit mRNAs (31), and denervation of rat diaphragm (31) and chicken leg muscle (32) raises the concentration of α-, γ-, and δ-subunit messengers (β-subunit mRNA was not measured in chicken muscle). In the mouse C2 cell line, the concentration of mRNAs encoding each of the four subunits increases significantly during fusion (31, 33).

We have found that TTX, 8-bromo-cAMP, and forskolin all increase the amount of α-subunit mRNA with little effect on γ- and δ-subunit messengers. These agents appear to differ from ARIA, however, in having a less pronounced effect on the concentration of the 285-nt fragment derived from a presumed nuclear precursor of α-subunit mRNA. These observations suggest that the action of ARIA is not mediated by muscle inactivity or by intracellular cAMP. Indeed, the effects of ARIA and TTX on the rate of AcChoR incorporation are additive, suggesting that they act by different mechanisms (15). Indirect evidence has been presented that calcitonin gene-related peptide (CGRP) increases the amount of α-subunit mRNA in chicken myotubes by a cAMP-mediated mechanism (34).

We have focussed our attention on the purification and mechanism of action of the 42-kDa ARIA with the thought that this molecule plays a role in the accumulation of AcChoRs at developing neuromuscular junctions. The dramatic and selective effect of ARIA on the amount of α-subunit mRNA is consistent with an important physiological function for this molecule. There is evidence that the density of receptors at newly formed junctions is controlled by localized insertion of receptors as well as by the trapping of receptors that have diffused from elsewhere on the surface of the muscle fiber (4–7). Our results suggest that ARIA acts by the first mechanism and raise the possibility that motor neurons alter the expression of specific genes in muscle cells during synaptogenesis. A number of proteins in addition to the AcChoR have been localized to the neuromuscular junction (35–39), and it is attractive to think that ARIA regulates the synthesis of one or more of them, perhaps by coordinately altering transcription of the genes that encode them.

We gratefully acknowledge Marc Ballivet for his generous gift of the chicken AcChoR clones. We also thank Cindy Nettrour and Jayne Collier for excellent technical assistance and John Merlie for a critical reading of the manuscript. This work was supported by a Clinical Investigator Development Award (D.A.H.) and a Physician-Scientist Award (D.L.F.), both from the National Institutes of Health, and by a McKnight Research Award and Grants from the Public Health Service (NS-18458) and the James S. McDonnell Foundation (G.D.F.).

1. Fischbach, G. D., Frank, E., Jessel, T. M., Rubin, L. L. & Schuetze, S. M. (1979) *Pharmacol. Rev.* **30**, 411–428.
2. Salpeter, M. M. & Loring, R. H. (1985) *Prog. Neurobiol.* **25**, 297–325.
3. Schuetze, S. M. & Role L. W. (1987) *Annu. Rev. Neurosci.* **10**, 403–457.
4. Anderson, M. J. & Cohen, M. W. (1977) *J. Physiol. (London)* **268**, 757–773.
5. Kuromi, H., Brass, B. & Kidokoro, Y. (1985) *Dev. Biol.* **109**, 165–176.
6. Ziskind-Conhaim, L., Geffen, I. & Hall, Z. W. (1984) *J. Neurosci.* **4**, 2346–2349.
7. Role, L. W., Matossian, V. R., O'Brien, R. J. & Fischbach, G. D. (1985) *J. Neurosci.* **5**, 2197–2204.
8. Jessell, T. M., Siegel, R. E. & Fischbach, G. D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5397–5401.
9. Bauer, H. C., Daniels, M. P., Pudimat, P. A., Jacques, L., Sugiyama, H. & Christian, C. N. (1981) *Brain Res.* **209**, 395–404.
10. Markelonis, G. J., Oh, T. H., Eldefrawi, M. E. & Guth, L. (1982) *Dev. Biol.* **89**, 353–361.
11. Buc-Caron, M.-H., Nystrom, P. & Fischbach, G. D. (1983) *Dev. Biol.* **95**, 378–386.
12. Godfrey, E. W., Nitkin, R. M., Wallace, B. G., Rubin, L. L. & McMahan, U. J. (1984) *J. Cell Biol.* **99**, 615–627.
13. Neugebauer, K., Salpeter, M. M. & Podleski, T. R. (1985) *Brain Res.* **346**, 58–69.
14. Knaack, D., Shen, I., Salpeter, M. M. & Podleski, T. R. (1986) *J. Cell Biol.* **102**, 795–802.
15. Usdin, T. B. & Fischbach, G. D. (1986) *J. Cell Biol.* **103**, 493–507.
16. Merlie, J. P. & Smith, M. M. (1986) *J. Membr. Biol.* **91**, 1–10.
17. Devreotes, P. N. & Fambrough, D. M. (1975) *J. Cell Biol.* **65**, 335–358.
18. Cathala, G., Savouret, J.-F., Mendez, B., West, B. L., Karin, M., Martial, J. A. & Baxter, J. D. (1983) *DNA* **2**, 329–335.
19. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 191 and 202.
20. Melton, D. A., Krieg, P. A., Rebagliati, M. R., Maniatis, T., Zinn, K. & Green, M. R. (1984) *Nucleic Acids Res.* **12**, 7035–7056.
21. Shieh, B. H., Ballivet, M. & Schmidt, J. (1987) *J. Cell Biol.* **104**, 1337–1341.
22. Ballivet, M., Nef, P., Stalder, R., & Fulpius, B. (1983) *Cold Spring Harbor Symp. Quant. Biol.* **48**, 83–87.
23. Nef, P., Mauron, A., Stalder, R., Alliod, C. & Ballivet, M. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7975–7979.
24. Cohen, S. A. & Fischbach, G. D. (1973) *Science* **181**, 76–78.
25. Betz, H. & Changeux, J.-P. (1979) *Nature (London)* **278**, 749–752.
26. Dubinsky, J. M., Falls, D. L. & Fischbach, G. D. (1986) *Soc. Neurosci. Abstr.* **12**, 189.
27. Klarsfeld, A. & Changeux, J.-P. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4558–4562.
28. Olson, E. N., Glaser, L., Merlie, J. P., Sebanne, R. & Lindstrom, J. (1983) *J. Biol. Chem.* **258**, 13946–13953.
29. Olson, E. N., Glaser, L., Merlie, J. P. & Lindstrom, J. (1984) *J. Biol. Chem.* **259**, 3330–3336.
30. Mishina, M., Takai, T., Imoto, K., Noda, M., Takahashi, T., Numa, S., Methfessel, C. & Sakmann, B. (1986) *Nature (London)* **321**, 406–411.
31. Evans, S., Goldman, P., Heinemann, S. & Patrick, J. (1987) *J. Biol. Chem.* **262**, 4911–4916.
32. Shieh, B. H., Ballivet, M. & Schmidt, J. (1986) *Soc. Neurosci. Abstr.* **12**, 25.
33. Buonanno, A., Mudd, J., Shah, V. & Merlie, J. P. (1986) *J. Biol. Chem.* **261**, 16451–16458.
34. Fontaine, B., Klarsfeld, A. & Changeux, J.-P. (1987) *J. Cell Biol.* **105**, 1337–1342.
35. Salpeter, M. M. (1969) *J. Cell Biol.* **42**, 122–134.
36. Sanes, J. R. & Chiu, A. Y. (1983) *Cold Spring Harbor Symp. Quant. Biol.* **48**, 667–678.
37. Beam, K. G., Caldwell, J. H. & Campbell, D. T. (1985) *Nature (London)* **313**, 588–590.
38. Froehner, S. C., Gulbrandsen, V., Hyman, C., Jeng, A., Neubig, R. & Cohen, J. B. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 5230–5234.
39. Bloch, R. S. & Hall, Z. W. (1983) *J. Cell Biol.* **97**, 217–223.