Volume 239, number 2, 339–342 FEB 06483 November 1988

Tissue distribution of mRNAs encoding muscarinic acetylcholine receptor subtypes

Akito Maeda, Tai Kubo, Masayoshi Mishina and Shosaku Numa

Departments of Medical Chemistry and Molecular Genetics, Kyoto University Faculty of Medicine, Kyoto 606, Japan

Received 22 September 1988

The tissue distribution of the mRNAs encoding muscarinic acetylcholine receptors (mAChRs) I, II, III and IV has been investigated by blot hybridization analysis with specific probes. This study indicates that exocrine glands contain both mAChR I and III mRNAs, whereas smooth muscles contain both mAChR II and III mRNAs. All four mAChR mRNAs are present in cerebrum, whereas only mAChR II mRNA is found in heart.

Muscarinic acetylcholine receptor subtype; RNA blot hybridization analysis; (Exocrine gland, Smooth muscle, Heart, Cerebrum)

1. INTRODUCTION

The muscarinic acetylcholine receptor (mAChR) mediates a variety of cellular responses through the action of guanine nucleotide-binding regulatory proteins [1]. The primary structures of four molecularly defined mAChR subtypes, designated mAChR I–IV, have been elucidated by cloning and sequence analysis of the cDNAs or genomic DNAs [2–8]. The antagonist-binding properties of the individual mAChR subtypes expressed from the cloned DNAs in *Xenopus* oocytes [2,8,9] indicate that mAChR I–III correspond most closely to the pharmacologically defined M_1 (I), M_2 cardiac (II) and M_2 glandular (III) subtypes [10–12], respectively. The agonist-induced responses in *Xenopus* oocytes and mammalian cells expressing the individual mAChR species provide evidence that mAChR subtypes are selectively coupled with different effector systems, albeit not exclusively [2,9,13–16]. In order to gain a further insight into the molecular basis of the functional heterogeneity of the mAChR, we have now examined the distribution of the four mAChR mRNAs in exocrine glands, smooth muscles, heart and cerebrum, using RNA blot hybridization analysis with probes specific for the respective mRNAs.

Correspondence address: S. Numa, Departments of Medical Chemistry and Molecular Genetics, Kyoto University Faculty of Medicine, Yoshida, Sakyo-ku, Kyoto 606, Japan

Abbreviation: mAChR, muscarinic acetylcholine receptor

2. MATERIALS AND METHODS

Total RNA was extracted from tissues of pigs and male Wistar rats (~200 g body wt) by the guanidinium thiocyanate method [17], and poly(A)$^+$ RNA was isolated as in [18]. The yield of poly(A)$^+$ RNA during oligo(dT)-cellulose chromatography was 7–10% for all preparations. Samples of poly(A)$^+$ RNA were denatured with 1 M glyoxal and 50% dimethyl sulphoxide [19], electrophoresed on 1.5% agarose gels and transferred [20] to Biodyne nylon membranes (Pall). The hybridization and washing conditions were the same as in [21], except that the concentration of each probe was 5 ng/ml. The mAChR subtype-specific probes were restriction fragments corresponding to regions with unique amino acid sequences located between the putative transmembrane segments V and VI; for probing the porcine mAChR I mRNA, a restriction fragment carrying a 3′-noncoding sequence was also used. The restriction fragments used were the *Pst*I(678)/*Sac*II(1033) fragment or the *Bgl*II(1656)/*Sph*I(2227) fragment from plasmid pmACR84 [2] carrying the porcine mAChR I cDNA, the *Rsa*I(708)/*Sma*I(1142) fragment from plasmid pSPHM10 [9] carrying the porcine mAChR II cDNA, the *Rsa*I(784)/*Bal*I(1391) fragment from plasmid pSpmACR7 [8] carrying the porcine mAChR III genomic DNA, the *Aat*I(806)/*Bgl*I(1385) fragment from plasmid pSPRM3 [8] carrying the rat mAChR III genomic DNA [5] and the *Xho*I(786)/*Sma*I(1178) fragment from

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00145793/88/$3.50

plasmid pSPRM4 [8] carrying the rat mAChR IV genomic DNA [5]; restriction endonuclease sites are identified by numbers (in parentheses) indicating the 5′-terminal nucleotide generated by cleavage; nucleotide residues are numbered in the 5′ to 3′ direction, beginning with the first residue of the ATG triplet encoding the initiating methionine. The probes were labelled by nick-translation [22] with [α-^{32}P]dCTP, their specific activities being 1.8–2.1 × 10^8 cpm/μg.

3. RESULTS AND DISCUSSION

Fig.1 shows the results of blot hybridization analysis of poly(A)$^+$ RNA from various porcine (A–C) and rat tissues (D,E) using probes specific for the individual mRNAs encoding mAChR I (A), II (B), III (C,D) or IV (E). A single major hybridizable RNA species was observed with each probe. The estimated sizes of the major RNA species encoding porcine mAChR I, II and III, and rat mAChR III and IV were ~3000 [2], ~6100 [3], ~9000, ~4500 [6,24] and ~3300 nucleotides [6,24], respectively.

As shown in fig.1A, the ~3000-nucleotide porcine RNA species hybridizable with the mAChR I-specific probe was present in cerebrum (lane 1), lacrimal gland (lane 2) and parotid gland (lane 3), but was not detectable in trachea (lane 6), urinary bladder (lane 7) and heart (lane 8). A barely detectable signal was observed for small (lane 4) and large intestines (lane 5). This may indicate the presence of minute amounts of the mAChR I mRNA in intestines or may alternatively be attributable to the mAChR I mRNA derived from coexisting neural tissue. On the other hand, the ~6100-nucleotide porcine RNA species hybridizable with the mAChR II-specific probe was found in all smooth muscles examined (fig.1B, lanes 4–7) as well as in heart (lane 8) and cerebrum (lane 1), but not in exocrine glands (lanes 2,3). The ~9000-nucleotide porcine RNA species hybridizable with the mAChR III-specific probe was present in exocrine glands (fig.1C, lanes 2,3) and smooth muscles (lanes 4–7) as well as in cerebrum (lane 1), but was not detectable in heart (lane 8). Minor RNA species of smaller sizes observed with this probe may arise from polyadenylation at different sites or from alternative RNA splicing. The ~4500-nucleotide rat RNA species hybridizable with the mAChR III-specific probe showed a similar tissue distribution, being present in submandibular gland (fig.1D, lane 2), small intestine (lane 3), trachea (lane 4), urinary bladder (lane 5) and cerebrum (lane 1), but not detectable in heart (lane 6). The ~3300-nucleotide rat RNA species hybridizable with the mAChR IV-specific probe was found in cerebrum (fig.1E, lane 1), but not in submandibular gland (lane 2), smooth muscles (lanes 3–5) and heart (lane 6).

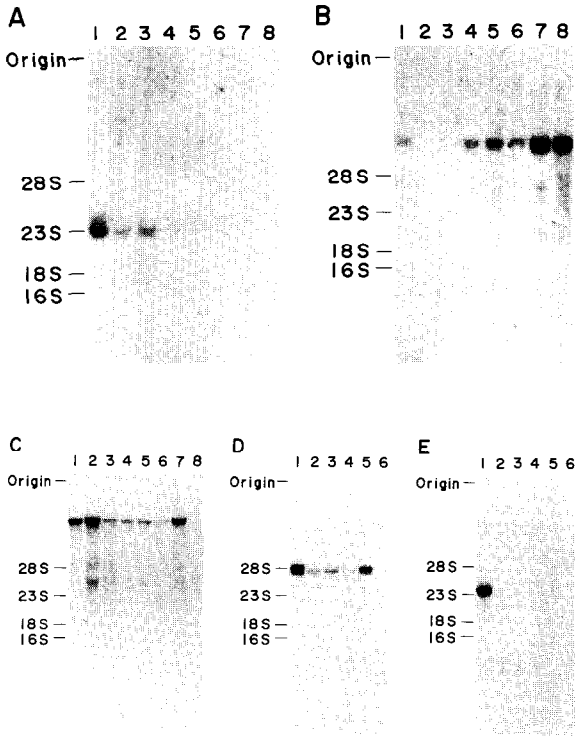

Fig.1. Autoradiograms of blot hybridization analysis of poly(A)$^+$ RNA from porcine (A–C) and rat tissues (D,E) using probes specific for the mRNAs encoding porcine mAChR I (A), porcine mAChR II (B), porcine mAChR III (C), rat mAChR III (D) or rat mAChR IV (E). (A–C) Analysis of poly(A)$^+$ RNA (15 μg each) from porcine cerebrum (lane 1), lacrimal gland (lane 2), parotid gland (lane 3), small intestine (lane 4), large intestine (lane 5), trachea (lane 6), urinary bladder (lane 7) and atrium (lane 8). (D,E) Analysis of poly(A)$^+$ RNA (15 μg each) from rat cerebrum (lane 1), submandibular gland (lane 2), small intestine (lane 3), trachea (lane 4), urinary bladder (lane 5) and heart (lane 6). Autoradiography was performed at −70°C for 70 h with an intensifying screen. The size markers used were porcine (or rat) and *Escherichia coli* rRNAs [23]. The mAChR I-specific probe used in the experiment shown in (A) was the restriction fragment derived from the 3′-noncoding region; similar results were obtained with the probe derived from the region between the putative transmembrane segments V and VI. For further details, see section 2.

The above results indicate that exocrine glands contain both the mAChR I and III mRNAs, whereas smooth muscles contain both mAChR II and III mRNAs. Exocrine glands and smooth muscles are representative sites for the pharmacologically defined M_2 glandular subtype of the mAChR [11,12]. Thus, the presence of mAChR III mRNA in both tissues conforms with the finding that mAChR III corresponds most closely to the M_2 glandular subtype in antagonist binding properties [8]. It has previously been reported that rat pancreas contains mAChR III mRNA, but not mAChR I mRNA [6]. The presence of all four mAChR mRNAs in cerebrum agrees with previous observations as does the presence of mAChR II mRNA in heart [2,3,5,6,24]. The differential tissue distribution of the four mAChR mRNAs observed in the present investigation strengthens our previous conclusion that the mAChR heterogeneity in tissues with respect to antagonist binding is attributable to the presence of individual molecularly distinct mAChR subtypes or various combinations of them [8].

Activation of mAChRs enhances secretion in exocrine glands and generally evokes contraction of smooth muscle [1]. Our results may indicate that the mAChR I and III subtypes are responsible for secretion in exocrine glands and that the mAChR II and IV subtypes are involved in contraction of smooth muscle. It has recently been shown that the mAChR I and III subtypes are coupled efficiently with phosphoinositide hydrolysis, whereas the mAChR II and IV subtypes are linked preferentially with adenylate cyclase inhibition [15,16]. Furthermore, the mAChR I and III subtypes expressed in NG108-15 neuroblastoma-glioma hybrid cells have been revealed to mediate inhibition of the M-current [16]. Most likely, activation of the mAChR I and III subtypes in exocrine glands enhances secretion at least partly through stimulation of phosphoinositide hydrolysis and consequent mobilization of intracellular Ca^{2+} [25]. The mAChR III subtype may mediate contraction of smooth muscle both through mobilization of intracellular Ca^{2+} induced by phosphoinositide hydrolysis [26] and through increased influx of extracellular Ca^{2+} resulting from inhibition of the M-current followed by membrane depolarization [27]. In view of our previous observation that the mAChR II subtype expressed in *Xenopus* oocytes activates principally Na^+ and K^+ currents [9], it is possible that this subtype is also involved in contraction of smooth muscle through activation of a nonselective cation channel [28].

Acknowledgements: This investigation was supported in part by research grants from the Ministry of Education, Science and Culture of Japan, the Institute of Physical and Chemical Research, the Mitsubishi Foundation and the Japanes Foundation of Metabolism and Diseases.

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