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Messenger RNAs coding for receptors and channels in the cerebral cortex of adult and aged rats

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Poly(A)$^+$ mRNAs from the cerebral cortex of aged (24 months) and young adult (3 months) rats were isolated and injected into *Xenopus* oocytes to express functional neurotransmitter receptors and voltage-operated channels. Electrophysiological recordings of induced membrane currents were used as a measure of the relative amounts of mRNA encoding different receptors and channels, and to study their functional properties. There were no large differences apparent between mRNAs from aged and adult rats, in marked contrast to the dramatic (1000-fold) changes in mRNA expression that occur during embryonic and postnatal development. The membrane currents induced by glutamate or acetylcholine (ACh) application were roughly one third smaller in oocytes injected with mRNA from aged cerebral cortex than in oocytes injected with mRNA from adult cerebral cortex, whereas currents induced by γ-aminobutyric acid (GABA), kainate or serotonin (5-HT) application, and by activation of voltage-operated Na^+ and Ca^{2+} channels were not significantly different. We did not observe any age-related differences in the properties of the receptors and channels studied.

INTRODUCTION

Many morphological and biochemical changes occur in the brain during aging. In the aged human brain there is an overall decrease in brain volume and weight[1,9] which is thought to be due to cell loss and cell atrophy in various brain regions. Cell loss is most marked in the substantia nigra (60% cell loss), but there is no change in the total number of neurons in the cerebral cortex[2,14,18,26]. In rodents, overall brain weight does not decrease, the cell loss in the substantia nigra is smaller (20%), and there is again little cell loss in the cerebral cortex[6,7,15]. In contrast to the loss of neurons, there is an increase in the number of glial cells in the human[37] and rat[2] cerebral cortex.

As well as these morphological changes, there are also changes in levels of neurotransmitters and their receptors in the aged brain[10,11,17,21,28,30,33,38]. However, the mRNAs coding for specific neurotransmitter receptors have not been previously studied during aging. We used the oocyte expression system[8,23,35] to examine age-related changes in the mRNAs coding for several types of neurotransmitter receptors and voltage-operated channels in the rat cerebral cortex. The largest changes that we detected between the aged and adult brain differed by a factor of less than two, in marked contrast to the dramatic changes seen during embryonic and postnatal development.

MATERIALS AND METHODS

Messenger RNA was isolated from Fischer strain 344 male rats at ages of about 3 months (young adult) and 24–26 months (aged), obtained from the National Institute on Aging Colonies at Charles River Breeding Laboratories. Procedures for mRNA extraction, injection into oocytes and electrophysiological recording were as described[4,5,19,35]. Agonists were bath-applied at the concentrations given in the legend to Fig. 1, and currents were recorded at a clamp potential of –60 mV. Unless otherwise noted, results are given as mean ± 1 S.E.M. When the data followed a Gaussian distribution, a Student's *t*-test was used to determine the significance of differences in expression of currents between adult and aged mRNA; otherwise the non-parametric Mann–Whitney *U*-test was used.

RESULTS

Yields of RNA and mRNA

The aged cerebral cortex yielded 0.029 ± 0.010 (S.D., 4 preparations) mg poly(A)$^+$ mRNA per gram wet weight of tissue. The corresponding value from adult cerebral cortex was 0.026 ± 0.003 (S.D., 3 preparations) mg/g. The amount of mRNA as a proportion of total RNA was slightly greater in the aged preparations, with aged preparations giving 6.6 ± 4.5% (S.D.) and adult preparations giving 4.0 ± 1.2% (S.D.), but this difference was not significant. The amount of total RNA per gram tissue was 0.54 ± 0.16 (S.D.) mg in aged preparations, and 0.68 ± 0.18 (S.D.) mg per gram tissue in adult preparations.

All mRNA preparations were tested in an in vitro

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translation system[5]. The translational activity was similar for mRNAs from aged and adult animals; expressed as a percentage of a standard value, the respective translational activities were 3.0 ± 1.9 (S.D.) and 2.7 ± 2.9 (S.D.).

mRNAs coding for neurotransmitter receptors

Injection of mRNA isolated from young adult or from aged cerebral cortex induced responses in oocytes to several neurotransmitters (Fig. 1) that were not present in non-injected oocytes. The peak sizes of these receptors were used as a measure of the relative amounts of mRNAs encoding specific receptors in the adult and aged cortex[4,5]. Mean values are summarized in Fig. 2A.

Glutamate-induced currents[13] were smaller in oocytes injected with mRNA from aged cerebral cortex (68 ± 10 nA; 78 oocytes) than with adult cerebral cortex (104 ± 20 nA; 42 oocytes) ($P < 0.05$; Mann–Whitney U-test). Currents evoked by NMDA (100 μM in the presence of 100 μM glycine) were also smaller in oocytes injected with mRNA from aged cerebral cortex (12.8 ± 3.3 nA; 30 oocytes) than from adult cortex (6.8 ± 1.3; 20 oocytes), but this difference was not significant (P = 0.05; t-test). The size of the response to kainate[13] did not differ appreciably between oocytes injected with adult (75 ± 10 nA; 81 oocytes) or aged mRNA (79 ± 7 nA; 81 oocytes). Furthermore, the reversal potentials (determined from current/voltage relationships) for the kainate induced responses were similar; –3.7 ± 2.1 mV (3 oocytes) with aged mRNA and –5 mV (1 oocyte) with adult mRNA.

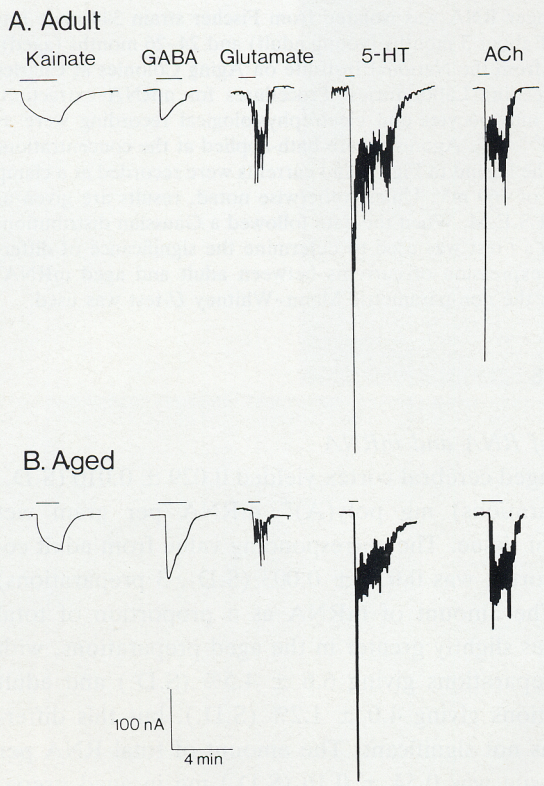

Fig. 1. Membrane currents elicited by various neurotransmitters injected with poly(A^+) mRNA from (A) adult and (B) aged rat cerebral cortex. Agonists were applied by bath superfusion for the time indicated by the bars, at the following concentrations: kainate, 10^{-4} M; GABA 10^{-3} M; glutamate 10^{-3} M; 5-HT 10^{-5} M; and ACh, 10^{-4} M.

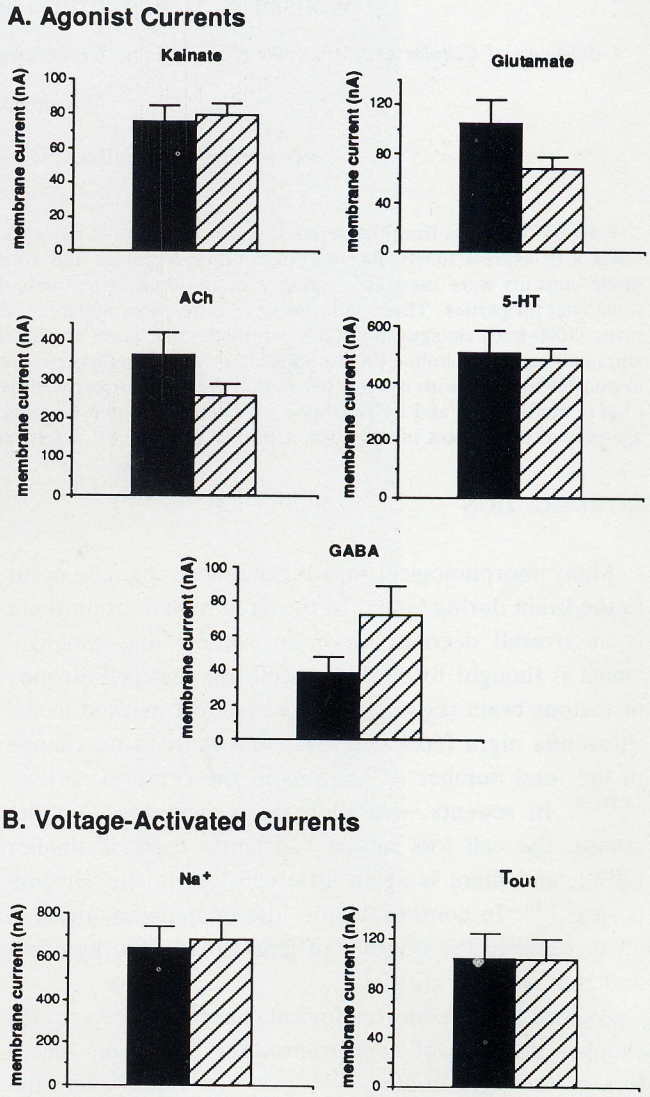

Fig. 2. Sizes of agonist- and voltage-activated currents induced in oocytes following injection with adult (black bars) and aged (gray bars) cerebral cortex mRNA. Each column represents the mean of 24–81 oocytes from 3 to 7 donors and error bars indicate S.E.M. For these experiments 4 aged mRNA preparations and 3 adult mRNA preparations were used. A: currents evoked by various agonists, applied at the same concentrations as in Fig. 1, and recorded at a clamp potential of –60 mV. B: voltage-activated currents. Na^+ currents were elicited by depolarization from –100 to –20 mV, and the values shown were obtained after subtracting passive currents evoked by the same depolarizations after addition of tetrodotoxin (300 nM) to the bathing solution. T_{out} currents were evoked by depolarization from –100 to 0 mV, and peak currents were measured with respect to the current remaining at the end of a 3 s depolarization.

In oocytes injected with mRNA from both ages, acetylcholine (ACh) and 5-hydroxytryptamine (serotonin or 5-HT) induced oscillatory chloride currents. Although responses to ACh are sometimes seen in non-injected oocytes[19], the present experiments were done using oocytes from donors with little or no native ACh response. The currents activated by ACh were significantly ($P < 0.05$, t-test) smaller in oocytes injected with aged mRNA (262 ± 31 nA, 75 oocytes) than in oocytes injected with adult mRNA (368 ± 59 nA, 42 oocytes). In both cases, the responses to 100 μM ACh were abolished by atropine (1 μM), indicating that they arose through activation of muscarinic receptors. Oocytes injected with mRNA from aged cerebral cortex gave a mean response to 5-HT of 481 ± 43 nA (81 oocytes), compared to 506 ± 78 nA (45 oocytes) with adult mRNA.

The mean response to γ-aminobutyric acid (GABA) was almost twice as large in oocytes injected with aged mRNA (72 ± 17 nA, 79 oocytes) than with adult mRNA (38 ± 10 nA, 45 oocytes) (Fig. 2); but this difference was not statistically significant ($P = 0.05$; Mann–Whitney U-test). The reversal potential of GABA-activated currents in oocytes injected with aged mRNA was –24 ± 7 mV (4 oocytes), similar to that (–22 mV) obtained with mRNA from young rats[4] and close to the chloride equilibrium potential in *Xenopus* oocytes[19]. The GABA-evoked current in oocytes injected with aged mRNA showed a rectification at hyperpolarized potentials, like that seen with mRNA from young animals[4]. Furthermore, the dose/response relations for GABA in oocytes injected with aged (3 oocytes) or adult (1 oocyte) mRNA were almost identical. At low doses the slopes of the relationships on double logarithmic coordinates (Hill coefficient) were about 1.3, and half-maximal responses were obtained with about 100 μM GABA. Responses evoked by glycine were smaller in oocytes injected with adult mRNA (1.9 ± 0.5 nA, 20 oocytes) than in oocytes injected with adult mRNA (3.7 ± 0.7 nA, 48 oocytes), but measurements of such small currents are subject to considerable error.

mRNAs coding for voltage-operated channels

Oocytes injected with brain mRNA express voltage-activated as well as agonist-activated channels[12]. Sodium currents were of similar sizes with adult mRNA (636 ± 103 nA; 26 oocytes) and aged mRNA (679 ± 88 nA; 37 oocytes) (Fig. 2B). The sodium currents induced by both mRNAs were blocked by tetrodotoxin (300 nM), and their inactivation rates did not differ; mean half-decay times were 2.18 ± 0.11 ms (24 oocytes) with adult mRNA and 2.35 ± 0.07 ms (33 oocytes) with aged mRNA.

Voltage-activated calcium channels were monitored indirectly by measuring the calcium-activated chloride current, T_{out}[22]. Because chloride channels are already present in large excess in native oocytes, the size of the T_{out} current gives a measure of the expression of exogenous calcium channels[5]. Fig. 2B shows mean currents after subtracting any small native T_{out} currents present in non-injected oocytes from the same donors. The current sizes in oocytes injected with adult or aged mRNA were almost identical (aged; 103 ± 17 nA, 35 oocytes; adult: 104 ± 21 nA, 24 oocytes).

DISCUSSION

The yields and translational activities of poly(A)$^+$ mRNAs from adult or aged cerebral cortex were not appreciably different. Thus, a simple interpretation is that changes in the size of the induced membrane current reflect changes in the amount of mRNA coding for that particular receptor[4,5]. However, the decreased ability of mRNA from aged brain to induce responses in the oocyte could also be due to differences in the translation of the mRNA, rather than differences in the amount of mRNA coding for a particular receptor[16]. For example, the mRNA from aged animals may be translated at a slower rate in the oocyte or the mRNA may contain errors which create non-functional receptors. We feel that these latter explanations are unlikely to account for our results, since the translational efficiencies of the mRNA measured in an in vitro system did not vary greatly and, although proteins have been shown to be altered in aged brains, this appears to be due to post-translational modifications rather than errors in translation[29,32].

Activation of induced 5-HT, ACh and metabotropic glutamate receptors produced oscillatory currents, due to the activation of the phosphoinositide second messenger system already present in native (non-injected) oocytes[24,25,34,36]. Thus, an increase in the size of these currents probably represents an increase in the expression of receptors, rather than increased expression of the second messenger system itself[5]. ACh-induced currents were smaller in oocytes injected with mRNA from aged animals than with mRNA from young adult animals, suggesting a decrease in the number of expressed receptors and hence in the amount of respective mRNA. In contrast, the size of the 5-HT response did not differ significantly between the two ages.

Our finding of a decrease in mRNA coding for muscarinic ACh receptor is compatible with results showing a decrease in radioligand (quinuclidinyl benzilate) binding in aged cerebral cortex[10,17,27,28,33]. Several studies of binding to 5-HT receptors have also shown a decrease with aging, in contrast to our finding of little change in mRNA levels. Specifically, labelled 5-HT binding decreases in aged human cerebral cortex[31], spiroperidol

binding decreases in aged rabbit cortex[38] and [^{3}H]ketanserin binding decreases in aged rat[3]. The oscillatory currents we recorded probably arise through 5-HT_{1C} receptors[20], so the discrepancy between our data and the binding studies may reflect differences between 5-HT receptor subtypes.

Glutamate-evoked responses were significantly smaller in oocytes injected with mRNA from aged rather than adult brain. A large part of the glutamate response is mediated by receptors coupled to the phosphoinositide messenger pathway, so the amount of mRNA coding for these metabotropic receptors[34] may be reduced in the aged cerebral cortex. However, other receptor subtypes are also activated by glutamate[13], and we were unable to separately assess the relative changes in each. Membrane currents activated by kainate were of similar size in oocytes injected with aged or adult mRNA, and the ionic selectivity of the expressed channels appeared not to change.

Currents induced by the inhibitory amino acid transmitters GABA and glycine were greater in oocytes injected with aged rather than adult mRNA. However, because of the small size and variability of the currents, the differences were not significant. Binding studies have shown little change in GABA receptor binding in the aged cerebral cortex[11,21]. The properties of GABA receptors expressed in the oocytes did not appear to change with age. With mRNA from both adult and aged cortex the receptor was associated with a chloride channel and the limiting slopes of the dose-response relationship suggested that at least two molecules of GABA may be needed to activate the channel (Hill coefficient of 1.3).

In oocytes injected with adult or aged mRNA, the size of the membrane currents induced by voltage-operated sodium and calcium channels were similar, suggesting that the respective levels of mRNAs coding for both these channels do not change with aging. Furthermore, we were unable to detect any differences in functional properties of the expressed sodium channels.

In conclusion, we used the oocyte translation system to examine the levels of mRNAs coding for receptors and channels during aging, and to study the functional properties of the expressed proteins. The largest changes that we detected in the aged brain differed from the young adult by only a factor of about two, in marked contrast to the dramatic changes in mRNA expression during embryonic and postnatal development, where levels increase and decrease a thousand-fold[4,5].

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