Alleviation of a Selective Age-Related Relational Memory Deficit in Mice by Pharmacologically Induced Normalization of Brain Retinoid Signaling

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Vitamin A and its derivatives, the retinoids, have been implicated recently in the synaptic plasticity of the hippocampus and might therefore play a role in associated cognitive functions. Acting via transcription factors, retinoids can regulate gene expression via their nuclear receptors [retinoic acid receptors (RARs) and retinoid X receptors]. In a series of experiments, the present study investigated the possible role of age-related downregulation of retinoid-mediated transcription events in the cognitive decline seen in aged mice. We observed that the brain (and hippocampal) levels of retinoid receptors and the expression of specific associated target genes were restored to presenescent (adult) levels in aged mice after acute administration (150 μg/kg, s.c.) of retinoic acid (RA). These effects of RA, however, could be abolished by the coadministration of an RAR antagonist. RA was also demonstrated to alleviate the age-related deficit in the CA1 long-term potentiation efficacy of aged mice *in vivo*. Moreover, RA was found to alleviate completely the performance deficit of aged mice to the control level in a two-stage spatial discrimination paradigm designed to assess relational memory. This promnesic effect of RA was again susceptible to abolition by RAR antagonist treatment. The parallel molecular, cellular, and behavioral correlates associated with the decrease of retinoid receptor expression and its normalization demonstrated here suggest that the fine regulation of retinoid-mediated gene expression is fundamentally important to optimal brain functioning and higher cognition. Specifically, a naturally occurring dysregulation of retinoid-mediated molecular events might be a potential etiological factor for cognitive deterioration during senescence.

Key words: cognitive aging; retinoic acid receptors; vitamin A; neurogranin; synaptic plasticity; LTP; learning; RAR; RXR

Vitamin A is a micronutrient with an unusually wide scope of biological actions that includes morphogenesis, vision, immune function, reproduction (Malik et al., 2000), and a major role in the fetal development of the nervous system (Maden et al., 1998). In the adult brain, the recent cartography of multiple nuclear receptors for the vitamin A metabolite retinoic acid (RA) by Krezel et al. (1999) has provided the basis for investigation into the potential role of vitamin A in the maintenance of mature nervous function (Malik et al., 2000). Retinoic acid receptors (RARα, β, and γ) and retinoid X receptors (RXRα, β, and γ) are DNA-binding proteins that, after activation by specific retinoid ligands, induce gene transcription by interacting with distinct promoter sequences in the target genes (Kastner et al., 1995). Knock-out studies have shown that these receptors (viz., RARβ and RXRγ) (Chiang et al., 1998) as well as certain target genes coding for neuronal proteins, such as neurogranin (RC3), a Ca^{2+}-sensitive calmodulin-binding protein (Iñiguez et al., 1994; Pak et al., 2000),

play a critical role in hippocampal long-term potentiation (LTP), the most widely studied form of synaptic plasticity thought to underlie information storage (McNaughton and Morris, 1987). A similar conclusion can also be derived from studies in mice deprived of vitamin A (Jacobs et al., 2000), and together the data strongly suggest a likely involvement of brain retinoid signaling in higher cognitive functions.

It has also been reported that the levels of mRNA for brain retinoid (RARβ and RXRβ/γ) nuclear receptors and the expression of certain target genes (including RC3) are reduced in aged mice by 20–30% relative to the levels in adults. Furthermore, such senescence-related reductions are susceptible to reversal by acute systemic RA (Enderlin et al., 1997). Prompted by these initial findings, the present study was undertaken to evaluate the extent to which the reduction in retinoid-related molecular signaling, despite being of moderate magnitude, might contribute to the cognitive and associated physiological alterations seen in aged mice.

Because previous studies have attributed to retinoid receptors an essential role in hippocampal synaptic plasticity, we first examined the effects of RA administration on the efficacy of LTP in CA1 induced by commissural stimulation *in vivo*. We then evaluated our principal hypothesis of an association between the downregulation of retinoid signaling and the emergence of a specific cognitive impairment in aged mice. To this end, we used a two-stage behavioral paradigm that can distinguish between the expression of declarative memory (which is impaired in senes-

cence) and the expression of procedural memory (which remains primarily intact in senescence) related to the same piece of learned material (Marighetto et al., 1999, 2000). This dissociation between the two forms of memory expression is also noted in human senescence, and the specific impairment in declarative memory is believed to stem from a dysfunction of the hippocampal region (Gabrieli, 1996).

Current theories suggest that declarative memory is critically dependent on the formation of a complex and coherent memory trace in which the configuration and inter-relationship among various aspects of past experience are represented (Johnson, 1992; Eichenbaum, 1997). Such complex relational representations enable two unique features of declarative memory expression: (1) the ability to compare and contrast separately acquired information and (2) the inferential use of past memories in novel situations (i.e., flexibility) (Cohen, 1984). The behavioral paradigm used here was specifically designed to assess these two features of declarative memory and is able to capture the selective declarative memory deficit of aged mice (Marighetto et al., 1999) as well as that of mice with hippocampal lesions (Etchamendy et al., 1999). Using this paradigm, we evaluated the potential promnesic effect of RA on declarative memory expression. In addition, we investigated whether such an effect could be antagonized by a blockade of retinoic acid receptors. If so, it would be consistent with the hypothesis that the promnesic effect of RA was mediated via an increase in the expression of brain (and hippocampal) retinoid receptors and their associated target genes in aged mice.

MATERIALS AND METHODS

Animals and drug preparation. The subjects were naive male mice of the C57BL/6 Jico inbred strain obtained from IFFA Credo (Lyon, France). They belonged to two distinct age ranges: ~21–23 months old (aged) and ~4–5 months old (adult). Mice of the latter age range served as presenescent controls. They were housed in a temperature-controlled and ventilated animal room under a 12 hr light/dark cycle. Mice destined for behavioral testing were placed on a restricted diet with their body weight maintained at 90% of their free-feeding level. All other mice were fed *ad libitum*.

Retinoic acid (Sigma, St. Louis, MO) and the RAR antagonist (CD3106; gift of Dr. U. Reichert) were dissolved in a vehicle solution containing polyethylene glycol, NaCl (0.9%), and ethanol mixed in a proportion of 70:20:10 by volume. CD3106 is also referred to as AGN193109 by Klein et al. (1996).

We used a dose of retinoic acid (150 μg/kg) that has been shown to be effective in reversing the age-related hypoexpression of brain retinoid signaling (Enderlin et al., 1997) and a dose of CD3106 (1 mg/kg) that is effective in blocking this reversal when coadministered. Both drugs were administered via the subcutaneous route.

In the electrophysiological study, three groups of mice of equal size ($n = 6$) were constituted: aged + RA, adult + vehicle, and aged + vehicle groups. They were injected daily with RA or vehicle solution for 4 d before high-frequency stimulation.

In the behavioral experiment, there were four groups of mice: (1) aged + RA group ($n = 10$; aged mice receiving an RA injection), (2) aged + RA + CD3106 group ($n = 6$; aged mice receiving injection of a mixture containing RA and the RAR antagonist CD3106) (Klein et al., 1996), (3) aged + vehicle group ($n = 8$; aged mice receiving a vehicle injection), and (4) adult + vehicle group ($n = 8$; adult mice receiving a vehicle injection). Daily injection began 4 d before behavioral testing commenced and continued until the end of behavioral testing. Injection was always made between 18:00 and 19:00 hr, whereas behavioral testing was conducted between 10:00 and 16:00 hr.

For reverse transcription (RT)-PCR analysis, two sets of subjects were used. The first set consisted of the mice that were used in the behavioral test and killed at the end of behavioral testing. Their hippocampi were submitted to RT-PCR analysis. The second set of mice was also divided into four groups ($n = 6$) according to age and drug treatment, similar to those in the first set. However, the second set of mice was never behav-
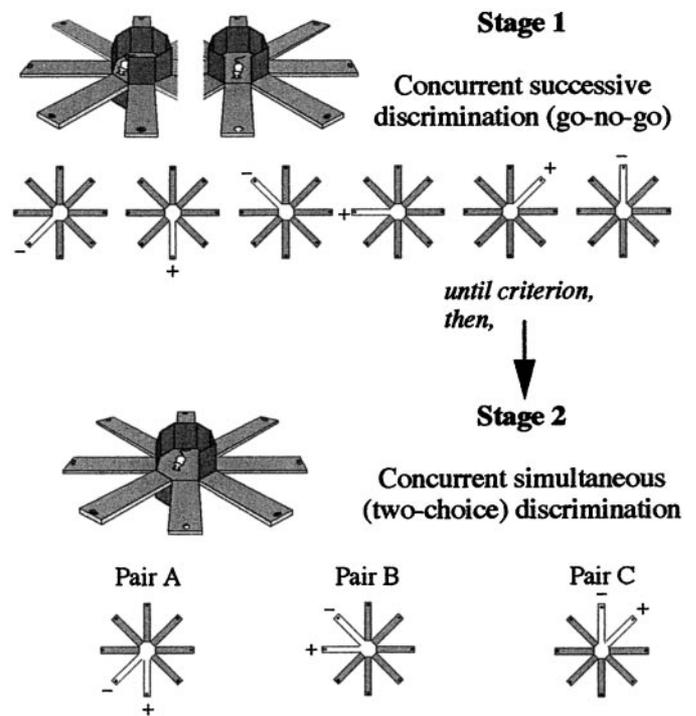

Figure 1. Design of the behavioral procedure.

iorally tested and was killed after 4 consecutive days of injection. Thus, the analysis of the second set of mice allows an evaluation of the effects of age and drug in naive subjects, free from any influence because of behavioral training. For the second (nontrained) set, RT-PCR analysis was performed either for the hippocampus alone or for the whole brain.

Learning and memory testing. The apparatus was a fully automated eight-arm radial maze, 150 cm in diameter, as described previously by Marighetto et al. (1999). The testing procedure is depicted in Figure 1. Before training on the discrimination task, the mice were habituated to the apparatus daily, with free access to all arms, for 2 d. For discrimination training, each subject was individually assigned a set of six adjacent arms. Of these, three served as positive (always baited) arms, and the remaining three served as negative (never baited arms).

Animals first acquired (stage 1) the valence or reward contingencies associated with each arm. For this purpose, subjects were tested continuously in a series of trials in which they were confronted with only one arm (either rewarded or not rewarded) open at a time. On each daily session, each arm was presented four times (i.e., 24 trials). Go–no-go discriminative performance was evaluated by the ratio between the median latency to enter nonrewarded (negative) arms and rewarded (positive) arms. A ratio above unity indicates that the mouse was more ready to enter rewarded arms than nonrewarded ones. Stage 2 began when performance based on this measure attained the criterion level. The criterion was defined as an overall ratio exceeding 1.5 over 2 consecutive days and exceeding 1.3 for each of the three pairs of arms to be designated at stage 2.

At stage 2, the reward contingencies remained unchanged, but the six arms were grouped into three pairs of adjacent arms of opposite valence (pairs A–C). In each trial, the subject was confronted with access to two adjacent arms of opposite valence (either of pairs A–C). A choice was considered to be made when the subject had reached the food well of an arm; this also triggered the closure of the door to the alternative arm. The trial was finished as soon as the subject returned to the central platform. Each daily session consisted of 20 consecutive trials comprising alternate presentations of pairs A–C according to a pseudorandom sequence. Two-choice discriminative performance was measured by the percentage correct (choice of the positive arm) and expressed as blocks of 2 d.

Biochemical analysis. The amounts of mRNAs coding for retinoic acid receptors (RARβ and RXRβ/γ), and for two protein products of their target genes, the tissue-type transglutaminase (tTG) (Chiocca et al., 1989) and RC3, were measured in the whole brain, as well as in the

hippocampus, for RXRβ/γ and RC3. The four groups of mice were killed either on the day after the fourth day of drug administration (i.e., before the beginning of behavioral training) or after the end of behavioral testing (i.e., after 25 d of drug administration). Their brain or hippocampi were removed and stored at −80°C. mRNAs were quantified by RT-PCR assay using β-actin as the internal control. The stability of β-actin mRNA levels (Dong et al., 1990; Rogue et al., 1993) was checked in all experimental conditions using a competitive RT-PCR method and the PCR MIMIC Construction Kit (Clontech, Palo Alto, CA). Total mRNA was extracted using the method described by Chomczynski and Sacchi (1987). cDNA preparation, PCR analysis, and quantitative determination of PCR products were performed as described previously by Alfos et al. (1996). Aliquots of the PCR reaction were sampled after each of the 7th through 24th amplification cycles. Amplification products were measured after their resolution by electrophoresis. The determination of the proportion of RARβ, RXRβ/γ, RC3, and tTG mRNA to that of β-actin was calculated according to the method of Chelly et al. (1990) using a semilogarithmic representation of the relative extent of amplification measured by counting the amount of incorporated ^{32}P. The oligonucleotide primers for RARβ, RXRβ/γ, tTG, and RC3 were as described previously by Enderlin et al. (1997).

In vivo *electrophysiology*. Mice were anesthetized with Avertin (10 ml/kg, i.p.). The stimulating and recording electrodes were made of two twisted pairs of platinum–iridium wires (90 μm in diameter) insulated except at the tip. By means of stereotaxic surgery, one electrode was positioned in the ventral hippocampal commissure (0.5 mm posterior to bregma and 0.3 mm lateral to the midline), and the other was in the contralateral pyramidal layer of CA1 (1.8 mm posterior to bregma and 1.3 mm lateral to the midline). The electrode positions were adjusted to maximize evoked field potentials, and then they were fixed with dental cement. Afterward, the animals were allowed to recover for 6 d. They were then habituated to the recording conditions for 3 d before recording sessions began.

CA1 field potentials evoked by single-pulse commissural stimulation (0.1 msec biphasic pulses) were recorded through junction field effect transistor operational amplifiers placed on the head of the animals and amplified, displayed on an oscilloscope, and recorded by a microcomputer for on-line averaging (each average was obtained with 20 responses at 0.2 Hz). The population spike was measured between the early positive peak and the large negative peak of the evoked potential. Stimulation intensity was adjusted to produce a population spike that was 30% of the maximum amplitude obtained from the baseline input–output curves. The baseline was established over a 2 d period (two recording sessions of 5 min/d). After the last 5 min baseline period, high-frequency stimulation (HFS) of the commissural path consisting of one train of 100 pulses was delivered at 100 Hz. The post-tetanus amplitudes of population spikes were followed every 5 min for 1 hr using the same single-pulse test that was used for the establishment of baseline.

After completion of the experiment, all mice were given an overdose of Avertin and perfused with saline (0.9%) followed by formol saline (10%). The exact placement of the electrodes was then verified by conventional histology.

Statistical analysis. Data were submitted to ANOVAs with the appropriate design. *Post hoc* comparisons were performed using the Scheffe *F* test.

RESULTS

Electrophysiological data

As shown in Figure 2*b*, a 1 sec train of HFS (at 100 Hz) of the ventral commissure induced a potentiation of the population spike in hippocampal CA1. LTP was evident in all groups, because the mean population spike amplitudes were significantly increased with respect to baseline (repeated measures followed by Scheffe *F* test, all *p* values < 0.05). However, the potentiation seen across time after HFS was significantly different among the three groups ($F_{(2,16)} = 3.86$; $p = 0.043$). Specifically, LTP seen in the aged + vehicle group was significantly weaker than that in the adult + vehicle group ($F_{(1,10)} = 4.99$; $p = 0.049$). This deficit in LTP was partially reversed in the aged mice treated with RA over the previous 4 d. Indeed, the mean population spike amplitudes seen across time after HFS were significantly increased in the aged + RA group compared with the aged + vehicle group
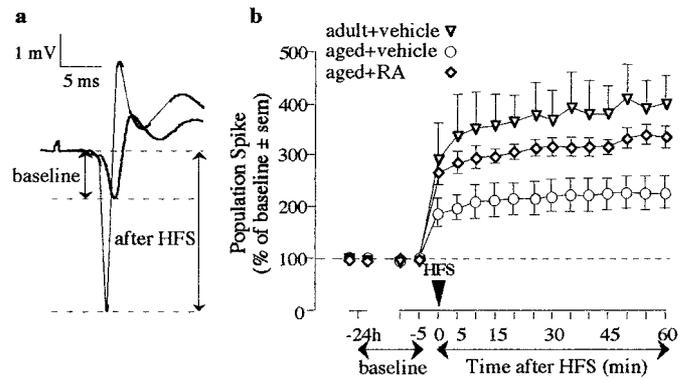

Figure 2. *a*, Representative records of the population spike in the CA1 region of the hippocampus before and after HFS of the contralateral ventral hippocampal commissure. *b*, The average amplitude of the population spike normalized to the average baseline value before HFS.

($F_{(1,11)} = 8.71$; $p = 0.013$) and were close to the amplitudes recorded in the adult + vehicle group.

Behavioral data

During stage 1 of behavioral testing, when the arms were presented one by one following a successive go–no-go discrimination procedure, neither the effect of age nor drugs significantly affected performance. There was no significant between-groups difference in the mean (±SEM) number of sessions needed to attain criterion performance (adult + vehicle, 10.25 ± 0.91; aged + vehicle, 9.75 ± 0.91; aged + RA, 11.60 ± 0.81; aged + RA + CD3106, 10.17 ± 1.05; $F_{(3,28)} = 0.89$; $p = 0.46$). Moreover, as shown in Figure 3, *left*, all groups displayed a similar response accuracy across the first and last six sessions as evaluated by measures of the no-go/go enter-latency ratio. Specifically, an ANOVA performed on this measure of performance across the first six sessions revealed no significant between-groups difference ($F_{(3,28)} = 0.39$; $p = 0.76$) with no significant improvement of performance across sessions ($F_{(5,140)} = 1.63$; $p = 0.16$) or group × session interaction ($F_{(15,140)} = 1.17$; $p = 0.30$). The same analysis performed across the last six sessions showed that all groups learned to distinguish between the positive (rewarded) and negative (nonrewarded) arms as evidenced by their progressive increased readiness to enter positive relative to negative arms. An ANOVA performed on these data yielded a significant effect of sessions ($F_{(5,140)} = 22.63$; $p < 0.001$) but no evidence of any between-groups difference ($F_{(3,28)} = 0.13$; $p = 0.94$) or group × session interaction ($F_{(15,140)} = 0.32$; $p = 0.99$). Thus, all groups were performing at a similar level (group, $F_{(3,28)} = 0.07$; $p = 0.97$) with a mean enter-latency ratio between 2.19 and 2.40, which was significantly above chance ($p < 0.001$ for each group), over the last two training sessions preceding stage 2 (Fig. 3, *middle*).

In contrast, significant between-groups differences emerged ($F_{(3,28)} = 8.99$; $p < 0.001$) when the mice were confronted with the three simultaneous discriminations composed of the six arms that were presented individually to them in stage 1 (Fig. 3, *right*). The vehicle-treated aged mice failed to translate their acquired preference for single arms into a choice between one positive and one negative arm presented as an explicit pair. With a mean percentage correct rate of 50.4%, they were performing significantly poorer (*post hoc* comparison, $p < 0.001$) than were adult controls that attained a level of 72.2% correct. Remarkably, RA-treated aged mice showed no sign of such a deficit (averaged at 68.4% correct) and significantly outperformed vehicle-treated
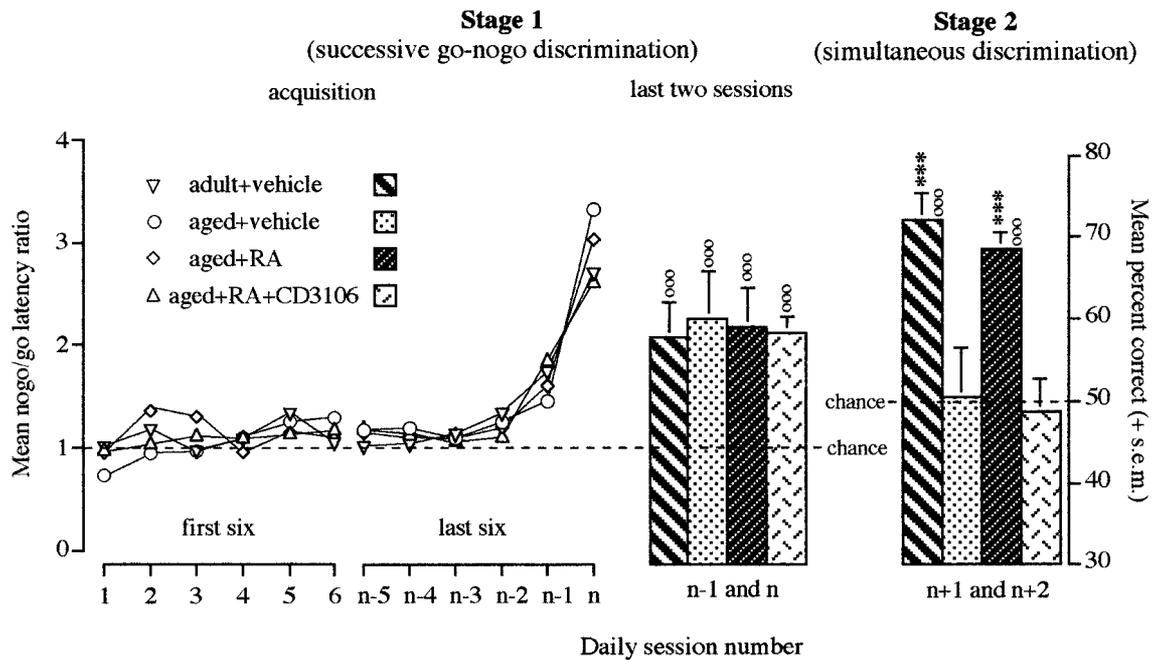
Figure 3. *Left,* Progression of the go–no-go discriminative performance evaluated by the no-go/go enter-latency ratio over the first six (1–6) sessions (i.e., the presently observed minimum number of sessions required to attain the criterion) and the last six ($n - 5$ to n) sessions of training before reaching criterion in stage 1. *Middle,* Mean no-go/go enter-latency ratio over the last two sessions ($n - 1$, n) of stage 1. *Right,* Two-choice discriminative performance expressed as the mean percentage correct over the two daily sessions of stage 2 ($n + 1$, $n + 2$). *Post hoc* Scheffe test: ***$p < 0.001$ versus aged + vehicle group; °°°$p < 0.001$ versus chance (50%).

aged mice (*post hoc* comparison, $p < 0.001$). Furthermore, this promnesic effect of RA was entirely abolished by the coadministration of the RAR antagonist CD3106. The aged + RA + CD3106 group attained an average correct rate of 48.7% and was not different from the aged + vehicle group (*post hoc* comparison, $p = 0.99$).

Biochemical data

One-way ANOVAs performed on biochemical data relative to the whole brain of mice submitted to 4 d of treatment revealed significant between-groups differences (all $F_{(3,11)} > 9.74$; all p values < 0.05). The aged + vehicle group exhibited significantly lower levels of brain mRNAs coding for retinoid receptors (RARβ, −27%; RXRβ/γ, −21%), for tTG (−26%), and for RC3 (−20%) relative to the levels of the adult + vehicle group (*post hoc* comparisons, all p values < 0.05). On the other hand, the mRNA contents in the aged + RA group were significantly increased relative to their age-matched controls (*post hoc* comparisons, aged + RA vs aged + vehicle, all p values < 0.05) and were at a level comparable with those seen in adult mice (*post hoc* comparisons, aged + RA vs adult + vehicle, all p values > 0.05). This RA-induced normalization of mRNA contents was almost completely blocked by the coadministration of the RAR antagonist CD3106 (*post hoc* comparisons, aged + RA + CD3106 vs aged + vehicle, all p values > 0.05; aged + RA + CD3106 vs aged + RA, all p values < 0.05) (Fig. 4a). A similar pattern of results was observed in the hippocampus (Fig. 4b; all $F_{(3,11)} > 26.1$; all p values < 0.001).

The biochemical data derived from the behaviorally tested mice conformed to the results described above (all $F_{(3,11)} > 8.38$; all p values < 0.008). This indicated that, irrespective of the time of death, vehicle-treated aged mice displayed lower levels of mRNAs coding for either RXRβ/γ (−39%) or RC3 (−31%)

relative to the levels of the adult + vehicle group. Two-way ANOVAs incorporating data from both sets of mice yielded an overall main effect of age for both variables [RXR, $F_{(1,8)} = 55.39$; $p < 0.001$; RC3, $F_{(1,8)} = 137.44$; $p < 0.001$] with no significant interaction between age and time of death [RXR, $F_{(1,8)} = 1.46$; $p = 0.26$; RC3, $F_{(1,8)} = 1.15$; $p = 0.31$]. RA administration alone almost totally abolished the age-related decrease in mRNA contents [effect of treatment, RXR ($F_{(2,12)} = 20.62$; $p < 0.001$) and RC3 ($F_{(2,12)} = 31.12$; $p < 0.001$)], and this effect was again similar regardless of the time of death [treatment × time of death interaction, RXR ($F_{(2,12)} = 0.05$; $p = 0.95$) and RC3 ($F_{(2,12)} = 0.41$; $p = 0.67$)]. Finally, this reversal effect of RA was totally blocked by the coadministration of CD3106 (*post hoc* comparisons, aged + RA + CD3106 vs aged + RA, p values < 0.05; aged + RA + CD3106 vs aged + vehicle, p values > 0.05) (Fig. 4b).

DISCUSSION

Long-term potentiation

First, the present data showed that the potentiation of the CA1 population spike induced by HFS of the ventral commissure was reduced in aged mice. This deficit is in agreement with a previous experiment using a similar brief HFS protocol to induce LTP (see Geinisman et al., 1995). Second, we demonstrated that this age-related deficit in LTP amplitude was ameliorated by the administration of RA. Because the dose of RA used here was also effective in restoring the 30% age-related decrease in retinoid signaling (as shown by the biochemical analyses) to the normal (adult) level, our data suggest that the initial moderate retinoid hypoexpression might be sufficient to produce the significant deficiency in synaptic plasticity seen in our vehicle-treated aged mice.
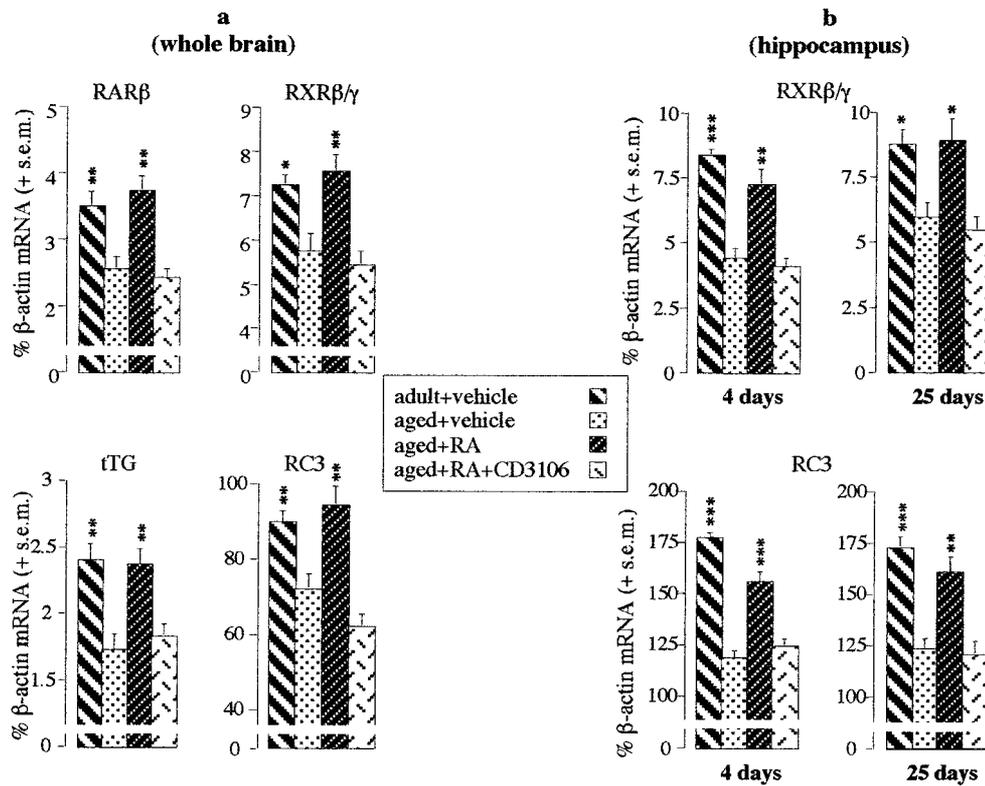
Figure 4. a, Whole-brain mRNAs in mice killed 24 hr after the fourth daily drug treatment. *b*, Hippocampal mRNAs in mice killed either 24 hr after the fourth daily administration of treatments (i.e., before the beginning of behavioral training) or after completion of testing in stage 2 (i.e., the day after the 25th daily administration of treatments). All values are expressed as the mean ± SEM of measures derived from three independent samples (*n* = 3). Each sample unit consisted of two pooled whole brains or two pooled hippocampi (i.e., a total of 6 animals). *Post hoc* Fisher tests: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; all significantly different from the aged + vehicle group.

In this respect, our data complement and further extend previous studies in knock-out mice that demonstrated, *in vitro*, an LTP deficit in mice not expressing RARβ, RARβ/RXRγ (Chiang et al., 1998), or RC3 (Pak et al., 2000). Our data suggest that normal mice with a natural, yet more limited, retinoid hypoexpression also exhibit significant functional alterations in hippocampal synaptic plasticity that can be reduced by pharmacological normalization of retinoid signaling. Thus, not only are retinoid-mediated transcription events involved in the molecular mechanisms underlying functional synaptic plasticity (as suggested by knock-out studies), but also a precise control in these nuclear events is a prerequisite for the functional integrity of such neural plasticity.

Effects of RA administration on the cognitive and associated retinoid-signaling dysfunction in aged mice

In sum, our biochemical and behavioral data show that (1) aged mice with a reduced expression of brain RARβ and RXRβ/γ mRNAs relative to those of adult mice were severely and specifically impaired in stage 2 of the discrimination task, (2) reversal of the age-related hypoexpression of brain retinoid receptors by RA administration was accompanied by a complete restoration of this behavioral impairment, and (3) the promnesic effect of RA was abolished by the coadministration of a selective RAR antagonist that concomitantly blocked the RA-induced normalization of retinoid signaling, hence demonstrating that this effect was mediated via brain retinoid receptors.

The selectivity of the cognitive deficit seen in our aged mice in stage 2 of the task as well as its reversal by RA renders unlikely the possibility that the amnesic effect of age and the promnesic effect of the drug are simply caused by their nonspecific effects on affect, motivation, perception, or motor control. Specifically, such nonspecific effects would have similarly influenced performance in both stage 1 and stage 2 because the basic requirements of the task were the same in both stages. It follows that the deleterious effect of age, and its reversal by RA, must be cognitive in nature. These contrasting effects should be taken as further evidence that these two versions of the position discrimination (i.e., stage 1 and stage 2) rely on distinct and dissociable memory systems (Etchamendy et al., 1999; Marighetto et al., 1999, 2000). The go–no-go discrimination in stage 1 involves separate unitary responses to individual arms and therefore can be achieved using elemental stimulus–response associations, forms of procedural and/or implicit memory that are independent of the hippocampal system. Conversely, the transfer to the two-choice problems at stage 2 entails comparisons between information acquired separately. Effective transfer would require the use of relational representations of past experiences and the functional integrity of the hippocampal formation (Eichenbaum et al., 1992).

Possible cellular and molecular mechanisms involved in the reversal of the age-related cognitive deficit by RA administration

The first line of explanation is based on the involvement of retinoid-mediated transcription events in synaptic plasticity. Because the formation of relational mnemonic representations is considered to be critically dependent on the Hebbian plastic properties of hippocampal synapses (Wallenstein et al., 1998), the observed promnesic effect of RA might stem from some restoration of the well documented age-related alterations in hippocampal synaptic plasticity (Barnes, 1994). In this respect, the RA-induced normalization of the age-related retinoid hypoexpression would reestablish a sufficient level of downstream-activated protein products for supporting the functional synaptic properties required for relational memory processing. RC3 is one of the numerous synaptic protein products of the retinoid-activated target genes [others include NMDA receptors (Younkin et al., 1993) or synaptophysin (Gaetano et al., 1992)]. Because the

content of these proteins in the brain is decreased via the aging process (Tamaru et al., 1991; Chen et al., 1998; but see Nicolle et al., 1999), they could well be implicated in the observed effects of RA here.

However, because of the large profile of genes the expression of which is under the control of nuclear retinoid receptors (Mangelsdorf, 1994), other related mechanisms could be considered as likely candidates for explaining our present results. Specifically, numerous studies have provided evidence that RA exerts a direct effect on the expression of a variety of cholinergic-specific proteins (Pedersen et al., 1995; Malik et al., 2000) and on the expression of neurotrophic factors (e.g., NGF) that, in turn, could be involved in the survival of cholinergic neurons (Korsching, 1993; Rylett and Williams, 1994; Corcoran and Maden, 1999). This is of particular interest because a decrease in central cholinergic neurotransmission has been one of the most consistently advanced hypothesis to account for the cognitive impairment associated with aging and certain age-related neurodegenerative diseases (Gallagher and Colombo, 1995).

Regardless of the cellular mechanisms involved, our results show that a 20–30% change in the expression of brain (and hippocampal) retinoid receptors is associated with significant alterations in certain target-gene mRNA contents, hippocampal LTP, and a hippocampal-dependent form of memory. These molecular, cellular, and behavioral correlates suggest that, although such alterations in retinoid signaling might be considered as moderate in magnitude, they can have significant consequences at various levels of brain function or organization. Our results thus point to retinoid hyposignaling as a potential cause of cognitive aging. This is consistent with recent data showing that a similar retinoid-signaling hypofunction, induced by a vitamin A-deprived diet in adult mice, also results in the same specific cognitive impairment as seen in our aged mice here (Etchamendy et al., 2000). Furthermore, this hypothesis is in line with the general principle "that the signals transduced by cells during growth and physiologic activity are the same as those that become overloaded during pathologic events and aging" (Malik et al., 2000). In other words, retinoids might play a role in the maintenance of phenotypic and functional properties of mature neurons, hence extending beyond their well documented role in neurodevelopment. It is noteworthy that dysregulation in retinoid transcription events has also been considered as a potential etiological factor in neurodevelopmental diseases such as schizophrenia (Goodman, 1998) or in age-related neurodegenerative disorders such as Alzheimer's disease (Connor and Sidell, 1997).

The present results as well as previous studies on the functional significance of retinoid signaling lead us to conclude that a precise control of the level of retinoid signaling is of fundamental importance; "dysregulated genes" implicated would provide a potential target for therapeutic intervention. Furthermore, intervention directed at the level of nuclear receptors would be expected to produce a more long-lasting effect than would other cognitive enhancers. The efficacy of such cognitive enhancers is usually limited by their narrow therapeutic time window. This advantage is illustrated in the present study, because RA was administered between 18:00 and 19:00 hr whereas behavioral testing was performed the next day between 10:00 and 16:00 hr. Finally, although traditional cognitive enhancers, such as acetylcholinesterase inhibitors (Mohammed, 1993), were designed to restore the deficiency of neurotransmission of a specific type, retinoid receptor-targeted drugs could potentially normalize a broad profile of gene expressions, thereby achieving a more global influence on the cellular homeostasis of the senescent brain.

Conclusion

The memory decline associated with normal aging is becoming a serious clinical issue among the populations of developed countries. Our study conducted in mice points to an altered regulation of the expression of selected genes as a potential cause of age-related cognitive impairment. Specifically, our data suggest that brain retinoid-signaling hypofunction deserves further consideration as a key potential target for curative therapeutic attempts.

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