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Age-Dependent Changes in Receptor-Stimulated Phosphoinositide Turnover in the Rat Hippocampus

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TANDON, P., W. R. MUNDY, S. F. ALI, K. NANRY, B. C. ROGERS AND H. A. TILSON. *Age-dependent changes in receptor-stimulated phosphoinositide turnover in the rat hippocampus.* PHARMACOL BIOCHEM BEHAV **38**(4) 861–867, 1991.—To study the changes in the hippocampal cholinergic system of chronologically old and behaviorally impaired animals, old (21 months of age) and young (3 months of age) male, Fischer-344 rats were used. The aged animals were tested on a reference memory task (Morris water maze) and found to be functionally impaired as compared to the young controls. Carbachol-stimulated phosphoinositide metabolism was measured in hippocampal slices from young and old rats. Slices were prelabeled with ^{3}H-inositol for 120 min and subjected to muscarinic stimulation in the presence of lithium. Following extraction of the slices with acidified solvent mixture, the inositolphosphates present in the aqueous fraction were isolated by ion exchange chromatography. Receptor-stimulated release of inositolphosphates (IPs) was found to be increased in the hippocampus of older animals. This age-related enhancement of IP release was in contrast to the decrease in choline acetyltransferase (CHAT) activity in the hippocampus. We postulate that alterations in the G-protein coupling with the muscarinic receptor leads to an increase in the phosphoinositide turnover in part as a compensatory mechanism for neuronal cell death and reduced transmitter levels.

Aging Phosphoinositide turnover Hippocampus Choline acetyltransferase Cholinergic muscarinic binding

IMPAIRMENT of cognitive functions such as learning and memory are a consequence of normal aging in humans (7). A similar age-related decline in cognitive function occurs in rats, particularly in those tasks requiring short-term memory or memory for spatial locations [e.g., (4, 29, 46, 60)]. Experimentally induced alterations in the functional integrity of the hippocampus appear to play an important role in the learning and memory deficits observed in young animals. Damage to the hippocampal formation following neurotoxicant treatment or lesioning disrupts performance on a wide variety of spatial tasks including those often used to assess age-related deficits (38, 41, 57). Thus, within the brain, the hippocampus becomes an important substrate for the studies of age-related alterations in neurotransmitter systems related to learning and memory deficits.

Among the neurotransmitter systems affected, the cholinergic system appears to be particularly vulnerable to changes occurring in selected brain regions during age-related neurodegenerative diseases, including senile dementia of the Alzheimer type. These disorders are often characterized by both motor and cognitive dysfunctions. Since similar but more subtle cognitive and motor abnormalities develop during normal aging it seems possible that alterations in the brain cholinergic systems may also occur with age. Although there is little direct evidence to support this hypothesis, previous research has shown that aging produces a number of changes in the cholinergic system (7). Reduction in muscarinic cholinergic neurotransmission has been reported in aged rats (50), while both extracellular (18, 34, 35) and intracellular (50) recording studies have revealed a highly selective reduction in the ability of acetylcholine (ACh) to increase excitability of hippocampal pyramidal cells. Changes in high affinity choline uptake in the hippocampus have also been reported in aged animals (21,52).

A number of studies indicate that age-related changes occur in muscarinic cholinergic receptor binding which are small, selective and localized in certain areas of the brain (2, 10, 49). However, the lack of changes in receptor binding may not necessarily reflect changes in receptor function (18). Receptor function may reflect alterations in effector-receptor coupling or receptor-mediator coupling leading to changes in the second messenger sys-

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tems, and thus to altered functional responses. The cholinergic and the noradrenergic systems are some of the many neurotransmitter systems that use inositol phosphates (IPs) as a second messenger in the signal transduction system. In the present study, agonist-stimulated hydrolysis of phosphoinositides was examined in the hippocampus of behaviorally impaired aged male Fischer-344 rats to determine whether the age-dependent changes in receptor function occur at the signal transduction level. Because of their possible importance in the interpretation of age-related changes in PI metabolism, we also determined other neurochemical parameters, including cholinergic receptor binding and levels of choline acetyltransferase (CHAT) activity in a similar group of animals.

METHOD

Animals

Male, Fischer-344 rats, obtained from Charles River Breeders (Raleigh, NC) were housed in groups of 4 in a room having a relatively constant temperature (20 ± 2°C) and relative humidity (50 ± 10%). The colony was maintained in a 12-h light/dark cycle with lights on at 0700 h. Laboratory chow (NIH diet 31) and tap water were continuously available. Rats were 3 months (young) or 21 months (old) of age at the start of the experiment.

Experimental Protocol

The ability of young and old rats ($N = 22$ per group) to acquire a spatial reference memory task was tested over a nine-day period, followed by a test for spatial retention. The next day, one set of rats ($n = 8$ per group) was sacrificed for regional measures of choline acetyltransferase (CHAT) and proteins. The second set of animals ($n = 8$ per group) was used to determine regional muscarinic binding, while the remaining animals ($n = 6$ per group) were used to study the turnover of phosphoinositides.

Spatial Navigation

The acquisition of a spatial navigation task was examined using a Morris water maze as modified by our laboratory (42). Animals were trained to swim to a platform hidden in a large circular pool (148 cm diameter × 60 cm height) located in a test room containing numerous extramaze cues. The pool was filled to a depth of 40 cm with water (28 ± 2°C) made opaque by powdered milk. The transparent platform was 10 cm in diameter and submerged 1.5 cm below the surface. Four equally spaced points around the edge of the pool (N,S,E,W) were used as start points and divided the pool into four quadrants.

Animals received 9 daily sessions of 4 trials per day in which rats were placed in the maze at one of the four starting points. On each test day all four start positions were used once in a pseudorandom sequence. There were approximately 5 min between trials. For each rat the platform was fixed in the center of one of the four quadrants and remained in that location for the duration of training. The latency to find the escape platform was recorded up to a maximum of 60 s. If a rat did not escape onto the platform within that time it was placed on the platform where it remained for 15 s.

To determine the extent of spatial retention, the tenth test day consisted of one 60-s "free swim" trial, during which the platform was removed from the pool. The time spent in the training quadrant (previously containing the platform) was recorded for each rat.

Choline Acetyltransferase (CHAT)

Rats selected for determination of CHAT activity were sacrificed by decapitation and the brains removed and dissected according to Glowinski and Iversen (23) into frontal cortex, striatum and hippocampus. The tissue was stored at −70°C for assay at a later time. Brain regions were assayed for CHAT using the procedure of Fonnum (19). Briefly, tissues were homogenized (5% w/v) in 10 mM EDTA, pH 7.4, using a sonic cell disrupter. The homogenates were activated with 0.5% (v/v) Triton X-100 to ensure release of enzyme. Ten μl of a substrate mixture [0.2 mM ^{14}C-acetyl-CoA (53 mCi/mmol, New England Nuclear, Boston, MA), 300 mM NaCl, 50 mM sodium phosphate buffer, pH 7.4, 8 mM choline bromide, 20 mM EDTA, pH 7.4, and 0.1 mM physostigmine] were added to 2 μl of the homogenate in a microtube and incubated at 37°C for 15 min. The microtube was placed in a scintillation vial and washed with 5 ml of sodium phosphate buffer followed by 3 ml of acetonitrile containing 10 mg of tetraphenylboron and 10 ml of toluene scintillant. The vials were shaken lightly and allowed to sit overnight before counting in a Beckman scintillation counter. Data were calculated as nmols acetylcholine/h/mg protein.

Receptor Binding Assay

Membrane preparation. For the binding assay, rats were decapitated, brains were removed on ice, digested and weighed. A crude membrane fraction was prepared by homogenation of tissue using a polytron homogenizer (Brinkman Instruments), in 20 ml volumes (1 g/20 ml) of 0.32 M chilled sucrose followed by centrifugation ($50{,}000 \times g$ for 10 min). The pellet was rehomogenized in distilled water (pH adjusted to 7.4), recentrifuged ($50{,}000 \times g$ for 10 min), suspended in 50 mM Tris-HCl (pH 7.4) buffer and centrifuged at the same speed and time. The final pellet was then suspended in the incubation buffer (50 mM Tris-HCl containing 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM KCl, 120 mM NaCl, 0.1% ascorbate and 10 μM pargyline, pH 7.4) at a concentration of 50 mg (wet weight equivalent)/ml (1,11).

[^{3}H]-Quinuclidinyl benzilate (QNB) binding. For QNB receptor binding, aliquots of membrane preparations from the frontal cortex, striatum, and hippocampus were incubated in 50 mM Tris-HCl buffer (pH 7.4) with 1.0 nM of [^{3}H]-QNB (29 Ci/mmol, New England Nuclear, Boston, MA) in duplicate. Incubation was carried out in duplicate for 60 min at 37°C in a total volume of 1 ml. Parallel incubations were performed in the presence of 1 μM atropine sulfate (Sigma Chemical Company, St. Louis, MO). After incubation, samples were diluted with 5 ml of ice-cold 50 mM Tris-HCl buffer and rapidly filtered under vacuum through Whatman GF/C glass fiber filters (Whatman Inc., Clifton, NJ). The filters were washed twice with 5 ml cold Tris-HCl buffer. This separation and washing procedure took less than 20 s. The filters were air-dried and placed into scintillation vials containing 10 ml of scintillation medium (Isolab Inc., Akron, OH). After vigorous shaking, the samples were stored overnight in the dark to minimize quenching, total radioactivity was quantified by liquid scintillation spectrometry (Tracor Mark III, Elk Grove Village, IL). Specific binding was calculated as the difference between the amount of [^{3}H]-QNB alone (total binding) and that in the presence of 1.0 μM atropine sulfate (nonspecific binding). The methods used were essentially the same as other filtration binding methods (62). However, it was necessary to establish the basic binding characteristics of the ligands used. Thus saturability, specificity and reversibility of different binding sites were delineated (1,11).

Protein determination. Aliquots of membrane preparations were used for the determination of protein content by the method of Lowry et al. (36). Protein determination for CHAT estimations was performed by a modification of the assay of Bradford (13)

using a commercially available kit (Bio-Rad, Richmond, CA). Bovine serum albumin (Sigma Chemical Co.) was used as the standard.

Phosphoinositide turnover. Phosphoinositide (PI) turnover was studied in hippocampal slices following incorporation of ^{3}H-inositol into slices and the subsequent release of inositolphosphates induced by agonist stimulation (9,24). Briefly, rats were decapitated and hippocampi removed. Krebs-Ringer bicarbonate buffer (KRB), saturated with O_2/CO_2 (95:5) (118 mM NaCl, 4.7 mM KCl, 0.75 mM $CaCl_2$, 1.18 mM KH_2PO_4, 1.18 mM $MgSO_4$, 24.8 mM $NaHCO_3$, 10 mM glucose, equilibrated by O_2/CO_2, 95/5, pH 7.4), was used for incubation and washing. Slices (350 × 350 μm) were cut and preincubated for 30 min, with repeated changes of buffer, in a shaking water bath at 37°C under a constant flow of O_2/CO_2. [^{3}H]Myo-inositol (Dupont, NEN, Boston, MA, spec.act. = 15 Ci/mmol) was added and the hippocampal slices were further incubated for 120 min for prelabelling. The slices were then washed three times with KRB (saturated with O_2/CO_2) and then incubated with various compounds for 30 min (unless otherwise stated), to stimulate agonist-induced IP release in the presence of lithium (8 mM). The agonists used were carbachol (10 μM), acetylcholine (100 μM), norepinephrine (10 μM) and 5-hydroxytryptamine (10 μM). Eserine (10 μM) was used as cholinesterase inhibitor with acetylcholine. Pirenzepine (1 μM), a muscarinic M_1 receptor antagonist, was used to determine receptor specificity of the effect. A mixture of chloroform/methanol/HCl (100:200:2) was used to stop the reaction. The water soluble inositol phosphates were separated over an anion exchange Dowex-1. Glycerophosphates were removed by ammonium formate (0.06 M) and Borax (0.005 M) solution (58). A fixed volume of the lower organic phase was taken, dried under nitrogen and counted for tritium in a Triton X-100 toluene scintillation fluid for the estimation of [^{3}H]-inositol incorporated into phospholipids.

In order to correct for possible differences in the volume of slices used or the degree of incorporation of [^{3}H]-inositol into phospholipids, data are calculated as dpm IP/(dpm IP + dpm phospholipid), and are expressed as a change from basal release. Basal release of IPs was the release of IPs without the addition of agonist, after incubation for 30 min at 37°C.

Separation of phospholipids and calculation of specific activity. The lower organic phase was separated and dried under nitrogen and resuspended in chloroform:methanol:water mixture (100:200:2; v:v:v). Phospholipids were separated by one-dimensional thin layer chromatography on 20 × 20 cm silica gel 60A, LK6D plates. Aliquots (10 μl) from each sample were spotted in duplicates. The solvent system used for separation was chloroform:methanol:20% methylamine (60:30:10 vol/vol) following the method of Bell et al. (8). The positions of the radiolabeled compounds were located by autoradiography. The area of silica gel containing PIP_2 was scraped and placed in scintillation vials containing 10 ml of toluene-based scintillation fluid and counted. In the duplicate set of plates, phospholipids were separated, identified and the appropriate areas scraped for the estimation of phosphorus (6). Specific activity of PIP_2 was calculated as μCi/μmol phosphorus.

Statistical Analyses

For data sets containing three or more groups, overall significance was determined using analysis of variance (ANOVA). If an overall treatment effect was observed, differences between groups were tested using Fisher's Least Significant Difference Test. Differences between two groups was determined using Student's *t*-test. The accepted level of significance was $p<0.05$.

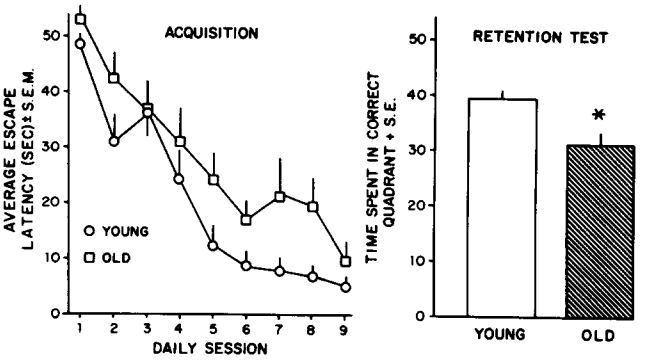

FIG. 1. Age-dependent acquisition of a spatial, reference memory task in the water maze. Young (3 months) and old rats (20 months) were given 4 training trials per day for 9 days. Data in left graph are average escape latencies per day ± S.E.M. for 8 rats per group. There was a significant effect of age (repeated measures ANOVA, $p<0.05$). On the tenth training day, the submerged platform was removed and the time spent in the training quadrant was recorded (right graph). *Significantly different from young animals (Student's *t*-test, $p<0.05$).

RESULTS

Spatial Learning

While both groups showed a decrease in escape latency with training, the old rats acquired the water maze task at a significantly slower rate than young controls (Fig. 1). In the free swim test for spatial retention on the tenth day of training (in which the submerged platform was removed), the old rats spent significantly less time in the quadrant formerly containing the platform compared to young rats.

CHAT Activity and ^{3}H-QNB Binding

Regional levels of CHAT were consistently lower (10–15%) in old rats compared to young rats (Table 1). Only in the striatum was the decrease in CHAT levels in old rats found to be statistically significant. Protein levels (mg/wet weight) were not changed in the brain regions tested in the old animals (data not shown).

^{3}H-QNB binding was measured in membrane preparations from the same brain regions of old and young rats. ^{3}H-QNB bind-

TABLE 1

REGIONAL LEVELS OF CHAT ACTIVITY AND ^{3}H-QNB BINDING

Region	Group	CHAT Activity nmol/h/mg Protein	^{3}H-QNB Binding fmol/mg Protein
Frontal Cortex	Young	70.1 ± 2.4	1313 ± 81
	Old	60.6 ± 4.5	1291 ± 55
Hippocampus	Young	46.5 ± 3.9	1132 ± 81
	Old	39.7 ± 4.6	1032 ± 74
Striatum	Young	138.0 ± 2.5	850 ± 86
	Old	114.6 ± 10.6*	675 ± 73*

Data are mean ± S.E.M. (n = 7–8/group).
*$p<0.05$ compared to young control.

TABLE 2

INCORPORATION OF ^{3}H-INOSITOL INTO LIPIDS AND SPECIFIC ACTIVITY OF PIP_2 IN HIPPOCAMPAL SLICES FROM YOUNG AND OLD RATS

Age	Incorporation (DPM/mg protein)	PIP_2 (DPM/mg protein)	Specific Activity of PIP_2 (μCi/μmol of phosphorus)
Young	6067.26 ± 461.11	700 ± 30.43	0.082 ± 0.015
Old	8532.60 ± 395.61*	903 ± 85.55	0.069 ± 0.011

Data are mean ± S.E.M. (n = 6/group).
*$p<0.05$ compared to young.

ing was decreased significantly in the striatum of old animals, with no significant difference observed in the hippocampus or frontal cortex compared to young rats.

Turnover of Phosphoinositides

^{3}H-Inositol incorporation into phospholipids. The incorporation of ^{3}H-inositol into lipids of hippocampal slices from young and old animals is shown in Table 2. There was a significant increase in the incorporation of ^{3}H-inositol into the lipid fraction of old animals. However, a statistically significant increase in labelling of the PIP_2 fraction was not observed in old animals. When the specific activity of PIP_2 fraction was determined, there was no difference between young and old rats.

Basal release. The study of endogenous release of IPs, i.e., release of inositolphosphate after 30 min of incubation at 37°C, without receptor stimulation showed no significant difference with age. In young control animals the basal release was 13.54 ± 0.43% of the total ^{3}H-inositol incorporated into the slices, while in old animals this value was 14.20 ± 0.33. The rest of the data (corrected for incorporation) was calculated as a % change from basal release.

Time course. To determine the effect of age on cholinergic muscarinic stimulation of inositolphosphates, a time course of carbachol-induced stimulation of phosphoinositide turnover was performed. As seen in Fig. 2, the carbachol agonist-stimulated turnover reached maximum level of stimulation in about 30 min. The old animals also showed maximum carbachol-induced stimulation of PI turnover at 30 min, with the magnitude of the response significantly greater than young controls.

Concentration-effect curve. To characterize further the interaction of cholinergic receptor-stimulated turnover of phosphoinositides between the old and the young animals, a concentration response curve was generated using carbachol as the agonist. Figure 3 shows that carbachol produced an increase in IP accumulation with a maximum response at 10 mM. In the old animals, the addition of carbachol produced a significant increase in PI turnover compared to the response in young animals. This effect was also observed when acetylcholine was used as the cholinergic agonist (Fig. 4).

Inhibition by antagonist. Pirenzepine, an M_1 antagonist, at a concentration of 1 μm inhibited ACh-stimulated PI turnover in both young and old animals (Fig. 4, insert). However, pirenzepine inhibited agonist-stimulated PI turnover more effectively (52% inhibition) in the young animals; old animals were less sensitive to the antagonist, as the same concentration of pirenzepine only produced 20% inhibition of ACh-stimulated PI turnover.

Stimulation with other agonists. To assess whether the increased magnitude of carbachol-stimulated inositolphosphate accumulation in old animals was due to a general hypersensitivity of the phosphoinositide turnover to receptor activation, the effect of acetylcholine, norepinephrine and 5-hydroxytryptamine was determined. Old animals showed a statistically greater stimulation of PI turnover in the presence of NE (Fig. 4) than young animals. However, 5-HT did not significantly stimulate PI turnover above basal levels in the hippocampus.

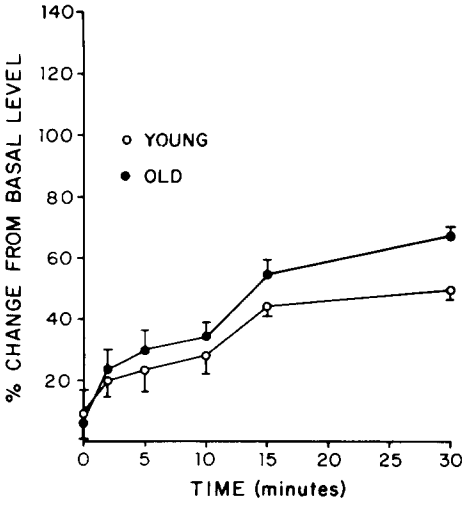

FIG. 2. Time-course of carbachol-induced accumulation of inositolphosphates IPs in hippocampal slices from young and old rats. The hippocampal slices were incubated in the presence of lithium (8 mM) with or without carbachol (10 mM) for different time periods. Results presented are means ± S.E.M. of at least three experiments done in triplicates. There was a significant effect of age (ANOVA, $p<0.05$).

DISCUSSION

The cholinergic system undergoes a continuous process of degeneration accompanied by biochemical and structural changes in

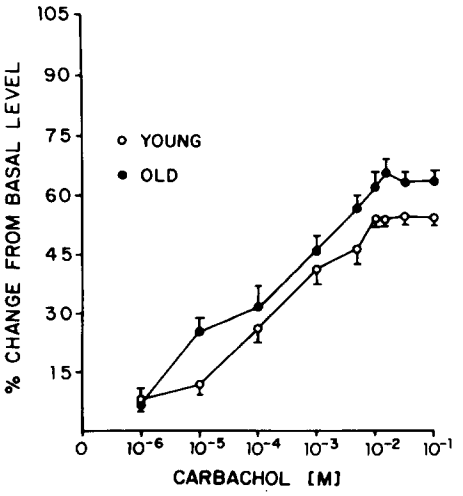

FIG. 3. Dose response of carbachol-induced accumulation of IPs in hippocampal slices from young and old rats. Results presented are mean ± S.E.M. of at least three experiments done in triplicates. Incubation time was 30 min. There was a significant effect of age (ANOVA, $p<0.05$).

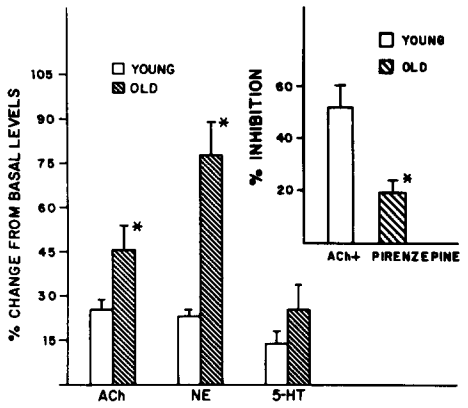

FIG. 4. Effect of various agonists on the accumulation of IPs in hippocampal slices. Acetylcholine (ACh) (100 μM) was used in the presence of eserine (10 μM). Norepinephrine (NE) and 5-hydroxytryptamine (5-HT) were used at a concentration of 10 μM. The incubations were done for 30 min. The rest of the procedure was the same as for carbachol-induced stimulation (see text). Data are mean ± S.E.M. of three experiments done in triplicate. Insert: Effect of pirenzepine (1 μM) on ACh (100 μM)-induced accumulation of IPs. Pirenzepine was added 15 min before the ACh. Incubations were carried out as described in the text. Results presented are mean ± S.E.M. from at least three experiments done in triplicates. *Significantly different from young animals (Student's *t*-test, $p<0.05$).

the remaining neurons during the aging process (17, 22, 27). Evidence suggests that these changes in the cholinergic system are related to memory impairment found during normal aging [(33–35); for review see (53)]. In the present study, 21-month-old Fischer-344 rats showed a spatial learning deficit in the water maze which was similar to learning impairments previously observed in the strain of rat (32,54). In addition, the old rats spent significantly less time in the training quadrant during the free swim test, indicating that these animals did not learn the spatial location of the escape platform as well as the young controls. Because the quadrant time is not a latency measure, the free swim test provides information regarding spatial learning that is largely unconfounded by changes in performance or motoric abilities which may occur in aged rats.

Age-related changes in cholinergic transmission in the rodent brain can vary with age, strain, and brain region. In aged Fischer-344 rats a significant reduction in CHAT activity, a presynaptic cholinergic marker, has been observed in the cortex (30), striatum (37,39) and hippocampus (37). However, other authors have failed to detect significant decreases in CHAT activity in the cortex (49), striatum (49) or hippocampus (33, 49, 52). In the present study, a significant decrease was observed only in the striatum. There are also conflicting reports regarding the effect of age on cholinergic muscarinic receptor binding. For example, in the cortex, there are reports of either no change (55) or a significant decrease (39, 44, 49) in [^{3}H]-QNB binding in aged Fischer-344 rats. [^{3}H]-QNB binding in hippocampal regions has been reported to be unchanged (44, 49, 55) or significantly decreased (39) with aging. Also in the striatum, there are reports of a significant decrease (39, 44, 55) or no change (49) in muscarinic receptors in aged Fischer-344 rats. As with CHAT activity, we observed a significant decrease in [^{3}H]-QNB binding in the striatum. Thus, strain and age differences, together with regional and possibly different dissection techniques for various brain regions, make it difficult to generalize age-related changes in cholinergic activity and receptor binding.

Guanine nucleotide binding regulatory proteins (G-proteins) are membrane bound proteins that transduce a variety of extracellular signals from cell surface receptors to intracellular effectors such as phosphoinositide turnover (25,26). G-proteins and receptors form high-affinity agonist binding complexes that are necessary for the stimulation of intracellular effector mechanisms, such as phospholipase C, by receptor agonists. Thus a change in receptor density cannot be taken as the sole determination of tissue responsiveness; the functional interaction of neurotransmitters and receptors with the G-protein must also be considered (51). Age-related changes in the binding properties of muscarinic receptors have not been associated with a change in the affinity constant of [^{3}H]-QNB (12, 14, 15, 35, 61), although a change in functional properties of the cholinergic muscarinic receptors and the coupling of receptors with enzymes involved in phosphatidylinositol hydrolysis during normal aging has been suggested (5). In the present study, muscarinic cholinergic agonists produced a significant increase in PI turnover in hippocampal slices from aged rats in the absence of significant changes in the affinity or density of muscarinic receptors in this brain region. Interestingly, increased PI turnover in aged rats was also observed when norepinephrine was used as the agonist, suggesting a general age-related increase in efficiency of postreceptor mechanisms. An increased efficiency of receptor-effector mechanisms in the aged rats is supported by the pirenzepine data (Fig. 4). Pirenzepine inhibition of PI turnover was significantly less in old rats compared to young rats at the same concentration of acetylcholine. These results are similar to the increase in carbachol and norepinephrine-stimulated PI turnover in cerebral cortical slices from aged rats reported by Nalepa et al. (43) and may represent a compensatory mechanism in response to reduced neurotransmitter levels. Other workers, however, have not observed increased receptor-mediated PI turnover in aged rats (56).

When compared with adults, brain tissue from either neonatal (3,28) or aged rats (present study) show a significant increase in the incorporation of ^{3}H-inositol into the total lipid fraction accompanied by an increase in agonist-stimulated release of IPs. This increase was apparent even when the amount of release was normalized for the increased incorporation in aged animals. This pattern may indicate age-dependent changes in receptor coupling to PI turnover (as discussed above) or could be indicative of preferential labeling of phospholipid pools which are recruited during receptor-mediated turnover. A large body of evidence indicates that the inositol phospholipids are not a metabolically homogeneous pool. Not only does each phosphoinositide show heterogeniety with respect to the degree of incorporation of lipid precursors (31, 40, 45), but also with respect to the release of IP upon stimulation (30, 31, 40, 45, 48, 59). In the context of the present study, the size of these pools may be altered in the aged animals or the specific activity of a minor (in amount) but more metabolically active pool of phosphoinositides is increased in the aged brain.

Alterations in inositol incorporation (as noted here) and in serine or ethanolamine incorporation [for review see (20)] may indicate age-related changes in membrane structure. It has been suggested that homoviscous adaptation is impaired with increasing age, causing a change in the membrane lipid fluidity in the brain (47). Changes in membrane fluidity can result in alterations in receptor function and modulate the action of many membrane-bound proteins. Changes in the cholesterol/phospholipid ratio of the hippocampus also occurs with age (16, 35, 47). Thus the changes observed in receptor function viz., alteration in agonist-stimulated IP release and change in pirenzepine-induced inhibition in aged rats, could possibly be linked to the age-related changes in membrane physiology observed by other workers. The alteration in membrane physiology occurring with age could pos-

sibly lead to structural alterations in the receptor or the receptor environment resulting in a change in receptor function coupling characteristics between receptor and the G-proteins.

More work needs to be done to elucidate these changes in membrane physiology occurring with age and its correlation with changes in receptor function observed in the aging brain.

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