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EFFECTS OF SUBCHRONIC ADMINISTRATION OF PYRITINOL ON RECEPTOR DEFICITS AND PHOSPHATIDYLINOSITOL METABOLISM IN THE BRAIN OF THE AGED MOUSE

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Summary—The effect of pyritinol, a commonly used nootropic drug, on receptor properties and function was investigated in different neuronal systems, possibly associated with age-related decline in brain function. Chronic treatment (15 days) of aged (22 months) female NMRI mice with pyritinol (200 mg/kg) restored the reduced density of *N*-methyl-D-aspartate receptors in the aged mouse brain. Furthermore, the total number of binding sites of the α_2-receptor ([^{3}H]yohimbine binding) decreased after treatment with drug, while the number of high-affinity agonist binding sites ([^{3}H]UK 14304 binding) was not changed. In both systems, receptor affinity was not influenced. The densities of other receptors investigated (muscarinic–cholinergic, benzodiazepine and β-adrenergic) were not altered by treatment with pyritinol. Additionally, the effect of pyritinol on phosphatidylinositol (PI) metabolism was investigated in dissociated neurones from young and aged mice. Muscarinic–cholinergic induced accumulation of phosphatidylinositol and the inositol phosphate response due to activation of G-protein by fluoride was increased in aged animals, treated with drug. The inositolphosphate response after stimulation with pilocarpine was slightly but not significantly increased. The metabolism of phosphatidylinositol in young animals was not altered by treatment with drug. These results support the hypothesis of a nootropic-mediated restoration of age-related brain deficits. Changes caused by pyritinol may be due to beneficial effects on age-related alterations of the properties of the neuronal membrane.

Key words—pyritinol, cognition enhancer, aging, receptor deficits, PI-metabolism.

The observation that cognition enhancers are much more effective in aged than in young animals certainly represents one of the most striking findings related to the pharmacology of these drugs. This observation has been reproduced many times and holds true for compounds belonging to different pharmacological subclasses of cognition enhancers (Porsolt, Roux and Lenegre, 1992). It suggests that the mechanism of action of cognition enhancing drugs might be associated with age-related deficits of the brain, relevant for cognitive functions. Although the biochemical basis of age-associated cognitive impairment is not fully understood, evidence from studies in animals and man suggest that deficits of the ageing brain, at the levels of muscarinic cholinergic (Müller, Stoll, Schubert and Gelbmann, 1991; Dewey, Volkow, Logan, MacGregor, Fowler, Schlyer and Bendriem, 1990) and *N*-methyl-D-aspartate (NMDA) receptor-mediated glutamatergic neurotransmission (Pelleymounter, Beatty and Gallagher, 1990; Kornhuber, Mack-Burkhardt, Konradi, Fritze and Riederer, 1989), might be of major importance. However, other neurotransmitter systems, possibly involved in the age-related decline of cognitive functions, include noradrenergic (Arnsten and Goldman-Rakic, 1985; Carlsson, 1987) and serotonergic (Gottfries, 1992) mechanisms and the benzodiazepine receptor as part of the γ-aminobutyric acid ($GABA_A$) synapse (Sarter and Stephens, 1988).

Pyritinol is one of the commonly used nootropic drugs. Its mechanism of action has been investigated in behavioural, electrophysiological and biochemical studies in man and animals, but no final conclusive hypothesis could be given. In old rats, chronic administration of pyritinol increases sensitivity to foot shock and spatial retention (Marston, Martin and Robbins, 1987). Additionally, pyritinol enhances passive avoidance retention in young and old animals (Marston *et al.*, 1987). Acute administration of the drug to young rats reveals a dose-dependent increase in levels of adenosinetriphosphate (ATP) in blood and enhances the age-related decreased utilisation of glucose in various regions of the brain (Greiner, Haase and Seyfried, 1988). Since, as mentioned above, deficits of cholinergic transmission have been particularly implicated in age-related alterations of memory and other cognitive functions, cholinergic parameters have also been the main target of investigations with pyritinol. Chronic treatment with pyritinol elevates the high-affinity uptake of choline in hippocampal and striatal synaptosomes, which is reduced by ageing (Greiner *et al.*, 1988; Pavlik, Benesova and Dlohozkova, 1987). Release of acetylcholine (ACh) is increased after chronic adminis-

tration of drug to old rats (Martin and Vyas, 1987), as well as the concentration of ACh in the striatum, cortex and hippocampus (Marston *et al.*, 1987; Martin and Vyas, 1987). Furthermore, pyritinol and some of its metabolites activate the release of ACh *in vitro*, dependent on their lipid solubility (Martin, Tucker and Widdowson, 1988).

So far, most of the investigations on pyritinol have concentrated on presynaptic mechanisms of central neurotransmission. Since previous work from this laboratory strongly suggests that a restoration of postsynaptic receptor–effector deficits of the ageing brain might be relevant for nootropic activity (Müller, 1988, 1992), the modulating effect of pyritinol on the properties of the *N*-methyl-D-aspartate (NMDA)-, muscarinic-, α_2-, β- and benzodiazepine receptors in the aged mouse brain were investigated.

Moreover, studies on receptor function were performed by measuring the inositolphosphate metabolism after stimulation of phospholipase C (PLC) with cholinergic agonists and fluoride. All of the systems investigated exhibit age-associated deficits in the aged mouse brain (Müller, Gelbmann, Cohen, Stoll, Schubert and Hartmann, 1992).

METHODS

Materials

Young (3 months) and aged (22 months) female NMRI-mice were obtained from Interfauna (Tuttlingen, Germany).

Radiochemicals (Table 1) were purchased either from Amersham Buchler (Braunschweig, Germany) ([^{3}H]*N*-methylscopolamine, [^{3}H]flunitrazepam and [^{3}H]dihydroalprenolol) or from NEN (Dreieich, Germany) (all others). Pyritinol (Encephabol®) was kindly donated from Merck (Darmstadt, Germany). All other chemicals were obtained from commercial suppliers.

Treatment

The animals were treated with pyritinol (200 mg/kg, daily, p.o.) for 15 days, where pyritinol was suspended in Methocel® solution (Methocel® 1% in 0.9% NaCl). Controls received Methocel® only. Twenty-four hours after the last treatment, the mice were killed by decapitation. The dose of pyritinol was chosen according to the most recent work done with the drug, measuring behavioural and biochemical effects in aged mice or rats (Marston *et al.*, 1987; Greiner *et al.*, 1988).

Binding assay

After decapitation, the brains were removed and quickly dissected on ice. Dependent on the receptor system investigated (Table 1), different areas of the brain were used: forebrain (including hippocampus), caudal brain (distal part of both hemispheres) (Gelbmann and Müller, 1991) or total brain, without cerebellum. Tissue was homogenized in 15 ml ice-cold

Table 1. Summary of binding assay conditions: receptor densities in distinct regions of the brain were determined by saturation experiments

Receptor	Ligand	Specific activity	Range	Blank	Area	Wet weight	Buffer	Incubation volume	Incubation time	Incubation temperature
Muscarine	[^{3}H]*N*-methyl-scopolamine ([^{3}H]NMS)	3.15 TBq/mmol	0.06–2.6 nmol/l	Atropine (10 μmol/l)	Forebrain Caudal-brain	1 mg/ml	Phosphate-buffer 50 mM, pH 7.4	300 μl	45 min	25°C
Benzodiazepine	[^{3}H]Flunitrazepam ([^{3}H]FNT)	3.11 TBq/mmol	0.02–2.0 nmol/l	Diazepam (10 μmol/l)	Forebrain Caudal-brain	2.5 mg/ml	Tris–HCl 50 mM, pH 7.4	500 μl	60 min	4°C
α_2 (total)	[^{3}H]Yohimbine	3.3 TBq/mmol	0.2–5.0 nmol/l	Phentolamine (10 μmol/l)	Total brain	11.5 mg/ml	Tris–HCl 50 mM EDTA 0.8 mM Ascorbic acid 0.8 mM NaCl 150 mM, pH 7.4	300 μl	30 min	25°C
α_2 (high affinity)	[^{3}H]UK-14304 [5-bromo-6(2-imidazoline-2-ylamino)-quinoxaline]	2.3 TBq/mmol	0.1–2.6 nmol/l	Phentolamine (10 μmol/l)	Total brain	11.5 mg/ml	see above + Catechol 0.3 mM Dithiothreitol 0.3 mM MgCl 10 mM NaCl *omit*	300 μl	70 min	25°C
β	[^{3}H]Dihydroalprenolol	1.59 TBq/mmol	0.3–7.5 nmol/l	Propranolol (10 μmol/l)	Caudal-brain	2.5 mg/ml 50 mM, pH 7.6	Tris–HCl	350 μl	30 min	25°C
NMDA	[^{3}H]MK-801	0.88 TBq/mmol	0.5–30 nmol/l	(+)MK-801 (100 μmol/l)	Forebrain	4 mg/ml	Tris–HCl 50 mM, pH7.4	500 μl	90 min	20°C

Data for K_D (dissociation constant) and B_{max} (maximal number of binding sites) were obtained from Scatchard plots by regression analysis

buffer, filled up to 40 ml and centrifuged for 25 min at 48,000 *g*. After discarding the supernatant, the pellet was washed twice (20 min, 48,000 *g*) and finally resuspended in the appropriate volume of buffer to adjust the tissue concentration (calculated as wet wt/ml) to the necessary range. The tissue-suspension was incubated with 50 μl of radioligand and 50 μl of the blank solution. For detailed conditions of the saturation experiments see Table 1.

Incubation was determined by rapid filtration through Whatman GF-B or GF-C filters, under slight vacuum. The filters were washed three times with ice-cold incubation buffer and placed in minivials. After extraction into 4 ml Quickszint 402 (Zinsser, Frankfurt, Germany), radioactivity was measured in a Beckman scintillation counter (counting efficiency 40%).

Data for K_D (dissociation constant) and B_{max} (maximal number of binding sites) were obtained from linear Scatchard plots by regression analysis. The concentration of protein was determined according to Lowry, Rosebrough, Farr and Randall (1951), using bovine serum albumin as standard.

Preparation of dissociated brain cells

Dissociated neurones were prepared according to Stoll and Müller (1991). After decapitation and removal of the cerebellum on ice, the brains were minced and suspended in ice-cold medium I (NaCl 138, KCl 5.4, Na_2HPO_4 0.17, KH_2PO_4 0.22, glucose 5.5 and sucrose 58.4, all mmol/l, pH 7.35). Tissue was dissociated by trituration through a nylon mesh (210 μm pore dia) with a pasteur pipette and filtered through a tighter nylon mesh (102 μm pore dia) by gravity. The resulting suspension (20 ml) was washed twice (400 *g*, 3 min, 4°C) in 20 ml medium II (NaCl 110, KCl 5.3, $CaCl_2$ 1.8, $MgCl_2$ 1, glucose 25, sucrose 70 and *N*-2-hydroxyethylpiperazine-*N'*-2-ethane-sulphonic acid (HEPES) 20, all in mmol/l, pH 7.4).

Stimulation assay for phospholipase C

The resulting pellet was resuspended in 6 ml medium III (like medium II, sucrose 40 mmol/l) or medium IV (like medium III, except $CaCl_2$ replaced by NaCl) for direct stimulation of phospholipase C by fluoride ions (F^-) (Jope, 1988) and incubated for 20 min in a shaking water bath (37°C).

To quantify inositol-phosphate metabolism, partly modified methods of Berridge, Dawson, Downes, Heslop and Irvine (1983) were used. After washing, dissociated neurones were resuspended in 6 ml medium III or IV (see above) and incubated with 25 μCi myo-[2-^{3}H(*N*)]-inositol (0.74 TBq/mmol) for 1 hr. Incubation-buffer was replaced by fresh, inositol-free medium and incubated for further 15 min (37°C, shaking water bath) with LiCl (final concentration 10 mmol/l). 460 μl aliquots were placed in plastic tubes containing 20 μl atropine solution (final concentration 10^{-5} mol/l) or 20 μl NaCl 0.9%, respectively, and incubated again for 10 min. (This step was omitted when NaF was used as the stimulating agent.) Stimulation was started by adding 20 μl of the agonist solution (carbachol or pilocarpine 1 mmol/l, NaF 20 mmol/l). After 60 min, stimulation was terminated by adding 200 μl $HClO_4$ 20% and sonication (10 min). All experiments were performed in triplicate.

Samples were centrifuged for 5 min at 10,000 *g* to separate precipitated protein and water-soluble metabolites. The residue was assayed for lipid extraction (see below), the aqueous phase was neutralised by precipitation of $KClO_4$ with KOH (10 mol/l, titrated against phenolred) and freeze dried after centrifugation. The residue was dissolved in 100 μl distilled water and applied on columns containing Dowex-1 chloride ion exchange resin, 1 × 8–200. The columns were washed with 5 ml water and 16 ml 5 mmol/l sodium tetraborate–60 mmol/l sodium formate to elute inositol and glycerophosphoinositol. Inositol-monophosphate ($InsP_1$) was eluted with 10 ml of 0.2 mol/l ammonium formate–0.1 mol/l formic acid. After a stimulation time of 60 min, no significant changes relative to baseline of bi- or polyphosphate concentrations were present (data not shown). Consequently, the remaining polyphosphates were coeluted with 12 ml or 1.8 mol/l ammonium formate–0.1 mol/l formic acid. The $InsP_1$ fraction was distributed in two vials, 13 ml Aquasafe 300 (Zinsser, Frankfurt, Germany) were added and the radioactivity was determined on a Beckman liquid scintillation counter. To account for different counting efficiencies, counts per minute (cpm) were transformed to dpm by quench correction. To extract the radioactivity of the lipid fraction, the pellet was sonicated in 900 μl chloroform–methanol (1.2), acidified with 250 μl HCl 2.5 N and further processed, according to Fisher and Agranoff (1980). After evaporation of chloroform, the remaining radioactivity was extracted in 4 ml Aquasafe 300 and determined as described above.

Stimulation was calculated as ratio of accumulation of $InsP_1$ to total incorporation ([^{3}H]$InsP_1$ + [^{3}H]lipids). Basal accumulation (atropine-, NaCl-values) was always subtracted.

Statistics

Binding assay: differences between groups were assessed using the unpaired Student's *t*-test. Inositolphosphate-metabolism: differences in responses were assessed by analysis of variance (ANOVA) (SAS-Institute, Cary, North Carolina) or by the unpaired Student's *t*-test. Differences were considered statistically significant for $P < 0.05$.

RESULTS

Receptor properties

Measurements of the possible changes of receptor properties, after treatment with pyritinol were carried out by saturation experiments to determine

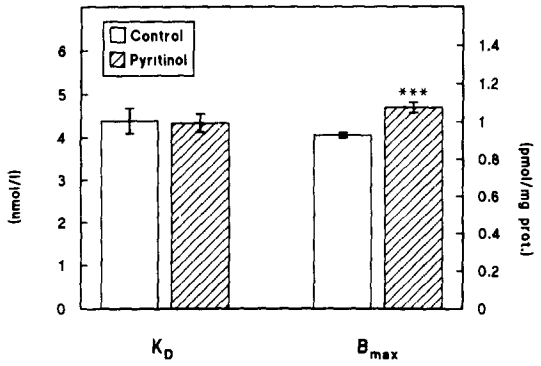

Fig. 1. Effect of subchronic (15 days) administration of pyritinol on the density of NMDA-receptors in the aged mouse forebrain. Mice (22 months) were treated with pyritinol (200 mg/kg, daily, p.o.) (▨) or vehicle (□). The K_D and B_{max} were determined as described under Methods. Data are means ± SEM of 6 experiments, each representing an individual animal. ***$P < 0.001$.

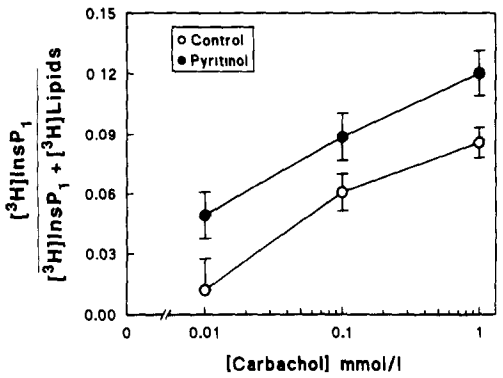

Fig. 2. Effect of subchronic administration of pyritinol on carbachol-induced accumulation of $InsP_1$ (treatment see Fig. 1). Dissociated neurones from treated (●) and control (○) animals (22 months) were stimulated for 60 min with increasing concentrations of carbachol, in the presence of lithium. Accumulation of $InsP_1$ was calculated as ratio of [^{3}H]$InsP_1$ to total incorporation. ([^{3}H]$InsP_1$ + [^{3}H]lipids). Data are means ± SEM of 8–16 determinations. Response after treatment was significantly increased over control (ANOVA, $P < 0.05$).

dissociation constants (K_D) and the maximum number of binding sites (B_{max}). In all the systems investigated, treatment with pyritinol did not change receptor affinities (Fig. 1, Table 2). On the other hand, treatment with pyritinol caused some specific alterations in receptor density (Fig. 1 and Table 2). The density of NMDA-receptors increased significantly from 0.93 to 1.08 pmol/mg protein after treatment with pyritinol in the forebrain of the mouse (Fig. 1). Furthermore, the total number of α_2-receptors ([^{3}H]-yohimbine binding) decreased significantly after administration of pyritinol, while the number of high affinity agonist binding sites was not changed (Table 2). Accordingly, the percentage of receptors in the high affinity conformation increased from 83.78 to 98.34% (Table 2). No changes in receptor density were found for the muscarinic and benzodiazepine receptor (forebrain or caudal brain) and for the β-receptor (caudal brain) (Table 2).

Phosphatidylinositol (PI)-metabolism

Carbachol-induced $InsP_1$-accumulation was determined in dissociated neurones from drug-treated and control aged animals. For all concentrations of carbachol (0.01, 0.1, 1 mmol/l) drug treated animals showed a larger response (Fig. 2). Comparison of both dose–response curves by ANOVA revealed a statistically significant effect of drug. Comparison of single values of treated and untreated animals indicated a large overlap between the two groups. However, small responses did not appear in the group of the treated animals, while very large values did not appear in the control group (data not shown).

The effects of treatment with pyritinol on carbachol-stimulated $InsP_1$-accumulation were specific for aged animals, since a similar treatment of young animals did not alter the dose–response curve for carbachol (Fig. 3).

Pilocarpine, a partial M_1-agonist, activated phosphoinositol-hydrolysis to a much lesser extent than carbachol (*ca* 30% of maximal stimulation), which is in agreement with other reports (Fisher, Klinger and Agranoff, 1983; Stoll and Müller, 1991). Again, pyritinol-treated aged animals showed a slightly increased response but the difference was not statistically significant, due to the high standard errors within the two groups.

Table 2. Effects of subchronic (15 days) administration of pyritinol on the density of several receptor systems

		K_D (nmol/l)		B_{max} (pmol/mg protein)	
Receptor	Area	Control	Pyritinol	Control	Pyritinol
α_2-Adrenergic (total)	Total brain	7.99 ± 2.75	5.98 ± 0.60	0.164 ± 0.022	0.132 ± 0.009*
α_2-Adrenergic (high affinity)	Total brain	1.83 ± 0.27	1.96 ± 0.15	0.136 ± 0.017 (83.78% of total binding sites)	0.130 ± 0.009 (98.34% of total binding sites)
Muscarinic	Forebrain	0.057 ± 0.004	0.057 ± 0.016	1.284 ± 0.066	1.301 ± 0.204
	Caudal brain	0.053 ± 0.004	0.049 ± 0.006	0.978 ± 0.048	1.031 ± 0.079
Benzodiazepine	Forebrain	0.820 ± 0.079	0.778 ± 0.068	0.907 ± 0.078	0.954 ± 0.067
	Caudal brain	0.792 ± 0.055	0.800 ± 0.079	1.025 ± 0.019	1.144 ± 0.014
β-Adrenergic $n = 6$	Caudal brain	0 930 ± 0 110	1 01 ± 0.18	0.088 ± 0.007	0 094 ± 0.006

For treatment conditions see Fig. 1. The K_D and B_{max} were determined as described under Methods. Data are means ± SD of 5 or 6 experiments, each representing an individual animal. *$P < 0.05$.

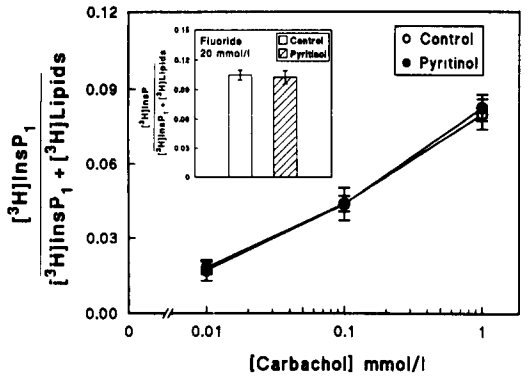

Fig. 3. Effect of administration of pyritinol to young mice (3 months, $n = 8$) on carbachol-induced accumulation of $InsP_1$. Inset: fluoride (20 mmol/l)-induced accumulation of $InsP_1$ from treated (▨) and control (□) young animals. For details see Fig. 2.

Addition of NaF (20 mmol/l) activated phospholipase C by direct activation of G-protein to a similar extent as carbachol (Fig. 4). Again, treatment with pyritinol caused a significantly greater response (Fig. 4). As in the former experiment, treatment with pyritinol of young animals under the same experimental conditions did not alter NaF-induced accumulation of $InsP_1$ (Fig. 3, inset).

DISCUSSION

At present, the mechanism of action of nootropics is not known but many hypotheses have been proposed to explain their common cognition enhancing properties. Müller (1988) suggested that a common mechanism of action of nootropics might be the restoration of age-related deficits of pre- and postsynaptic receptor–effector systems. This hypothesis is supported by the partial restoration of the age-related decline of muscarinic–cholinergic and NMDA-receptor density after treatment with piracetam (Pilch and Müller, 1988; Stoll, Schubert and Müller, 1991) or phosphatidylserine (Cohen and Müller, 1992; Gelbmann and Müller, 1991).

The present findings with pyritinol fit only partially with this hypothesis, since pyritinol increased the density of NMDA-receptors but had no effect on the density of muscarinic–cholinergic receptors. Thus, restoration of age-related deficits of central muscarinic cholinergic receptors is probably not a general property of nootropic drugs, as was previously believed (Müller, 1988). However, like piracetam and phosphatidylserine, treatment with pyritinol increased the density of NMDA-receptors in aged mice. The glutamatergic system is of major importance for cognition and learning (Morris, 1989) and disturbances of this system are probably involved in the pathophysiology of Alzheimer's disease (Marangos, Greenamyre, Penney and Young, 1987; Palmer and Gershon, 1990) and age-related cognitive deficits (Baskys, Reynolds and Carlen, 1990; Pelleymounter *et al.*, 1990). In aged animals, glutamatergic neurotransmission is impaired at the level of the density of NMDA-receptors (Cohen and Müller, 1992) and their function (Baskys *et al.*, 1990). The pyritinol-induced increase in density of NMDA-receptors could at least partially explain its cognition enhancing properties, as it has been suggested for phosphatidylserine (Cohen and Müller, 1992).

Pyritinol decreased the total number of binding sites of α_2-receptors and increased the ratio of high-affinity sites, to the total number of binding sites. Gelbmann and Müller (1990) found a specific loss of α_2-receptors in the high affinity state in the aged mouse brain, whereas no change was found in total number of binding sites. Accordingly, the present findings with pyritinol can only be interpreted with caution as a partial reconstitution of age-related changes of properties of α_2-receptors by increasing the percentage of α_2-receptors in the high affinity state.

Very little information is available on pyritinol-induced activation of postsynaptic receptor–effector coupling. Greiner *et al.* (1988) described an increased baseline level of c-GMP in distinct areas of the brain after chronic treatment with pyritinol which was explained as an effect of pyritinol on cholinergic transmission. However, the recent status of cGMP as a second messenger of central muscarinic cholinergic receptors, is still open to question and this will be even more the case for baseline levels for cGMP. As mentioned above, treatment with pyritinol did not elevate the density of muscarinic receptors in the aged mouse brain. However, the same treatment resulted in an activation of hydrolysis of phosphoinositol after stimulation with cholinergic agonists. Since pyritinol does not bind to the muscarinic receptor (Greiner *et al.*, 1988) and, as mentioned above, did not change the density of receptors, a direct effect of this drug on muscarinic cholinergic receptors, can therefore be ruled out. The activation of carbachol-induced hydrolysis of phosphoinositol could be due to a direct

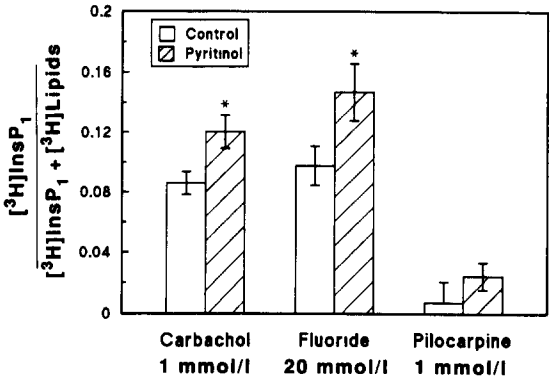

Fig. 4. Effect of subchronic administration of pyritinol to aged mice (22 months) on carbachol- (1 mmol/l, $n = 16$), pilocarpine- (1 mmol/l, $n = 8$) and fluoride- (20 mmol/l, $n = 8$) induced accumulation of $InsP_1$ in dissociated neurones from treated (▨) and control (□) mice (22 months) (for details see Figs 1 and 2). $^*P < 0.05$.

effect on postsynaptic receptor–effector coupling, which would facilitate the intracellular response, after receptor-activation. The increased phosphatidylinositol response in aged animals after stimulation with fluoride, as a consequence of the treatment with pyritinol, supports this hypothesis. Fluoride is thought to activate G-proteins by mimicking the γ-phosphate of GTP, in the form of the $[AlF_4]^-$ complex, which results in dissociation of the α-subunit of the γ-subunit of G-proteins, leading to a stimulation of phospholipase C (Jope, 1988). A better coupling of receptor, G-protein and phospholipase C might therefore be a possible explanation for the effects of pyritinol observed in the present study. This more effective receptor–effector-coupling could be due to restoration of changes in membrane composition and structure, which occur during ageing (Cohen and Zubenko, 1985; Viany, Cervato, Fiorilli and Cestaro, 1991), probably as a consequence of a reduced protection of the aged brain against free radicals. Since pyritinol seems to possess potent radical scavenger properties (Pavlik and Pilar, 1989), beneficial effects of pyritinol, at the level of the neuronal membrane, form an attractive hypothesis to explain these findings about the effects of this drug on the phospholipase C system and on receptor deficits of the ageing brain.

As mentioned above, comparison of single values of individual animals revealed that very low inositol phosphate responses in the control group were not seen in the pyritinol-treated group. Referring to the hypothesis of a nootropic-mediated restoration of age-related deficits and considering that the extent of age-related impairment may differ between individual animals, it might be speculated that especially severely impaired animals will profit from treatment with pyritinol. Since a cross-talk between the glutamatergic$_1$ and cholinergic systems has been shown (Baudry, Evans and Lynch, 1986), the combination of the present results, i.e. the increase in density of NMDA-receptors and the increase in muscarinic cholinergic receptor-mediated hydrolysis of phosphatidylinositol even further emphasise the possible relevance of these effects for the cognition-enhancing properties of pyritinol.

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