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Pyritinol facilitates the recovery of cortical cholinergic deficits caused by nucleus basalis lesions

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Summary. The effect of a nootropic, Pyritinol, on the recovery of cortical cholinergic deficits induced by injury of the nucleus basalis has been tested on two groups of unilateral quisqualic acid nbM-lesioned rats. The first group had a 30 nmol lesion producing a cortical cholinergic impairment at 21 days, with a spontaneous recovery at 45 days. The second group had a 50 nmol lesion that produced a deeper cholinergic deficit, which did not recover at 45 days. Pyritinol enhanced the recovery in the 30 nmol group of animals on the 21st day after surgery. The recovery was measured as an increase in the activities of acetylcholinesterase (AChE), choline acetyltransferase (ChAT) and the high affinity choline uptake system, and the histochemical densities of the cortical AChE network and the M_2 receptor. Histochemical analysis of the nbM enabled cortical recovery to be related to the number of surviving neurons and also to their hypertrophy and AChE-ChAT hyperactivity. Pyritinol enhanced recovery in 30 nmol lesioned animals but in the other group, with a lower number of surviving neurons and a lower ability of the cells to become hypertrophic, the drug was unable to promote cortical recovery.

Keywords: Experimental Alzheimer models, nucleus basalis, neocortex, cholinergic deficits, recovery, nootropics, pyritinol

Introduction

Disturbances of central cholinergic neurotransmission are involved with the decline of learning, memory and other cognitive functions during ageing (Bartus et al., 1982) and in the course of Alzheimer Disease/senile dementia of the Alzheimer type (AD/SDAT) (Collerton, 1986; Coyle et al., 1983; Davies, 1979; Deutsch, 1971; Perry et al., 1977, 1992; Robbins et al., 1989; Toledano, 1988). Thus various cholinomimetics have been utilized in the treatment of these disorders. However, only small therapeutic benefits have been obtained from these treatments (Haroutunian et al., 1985; Klein et al.,

1992; Palacios and Spiegel, 1986; Sarter, 1991). On the other hand, the use of certain nootropics (e.g. pyrrolidinone derivatives like piracetam, vinpocentine, etc), that have poorly defined actions on the cholinergic system, has proved useful in several clinical and experimental studies (Groo et al., 1987; Porsolt et al., 1988; Sarter, 1991; Schindler et al., 1984; Stoll et al., 1991). This is also true for Pyritinol (Herrmann et al., 1986, 1988; Hoyer et al., 1977; Knezevic et al., 1990; Marston et al., 1987; Seyfried, 1989). These various drugs have demonstrated differing effects on brain acetylcholine levels, acetylcholine release (basal, induced), high affinity choline uptake activity, cGMP levels, muscarinic receptor functionality, etc. However, their cellular and the subcellular targets in the cholinergic system and in the cognitive process have not been well defined up to now (Greiner et al., 1988; Martin and Vyas, 1987; Martin and Widdowson, 1990; Pepeu and Spignoli, 1989; Pilch and Müller, 1988; Seyfried, 1989; Shih and Pugsley, 1985). Although pharmacological effects on other non cholinergic systems (monoaminergic, aminoacidergic, peptidergic) have been rejected (Greiner et al., 1988) or considered to be minimal (Pepeu and Spignoli, 1989), other trophic or regulatory effects have been postulated in relation to corticosteroids and other factors (Mondadori and Petshke, 1987) and to phospholipid/cholinergic system interactions (Martin and Widdowson, 1988; Gelbmann and Müller, 1991).

In order to determine possible mechanisms of action of the nootropic Pyritinol, a study was carried out to determine its effect on recovery from quisqualic acid lesions of the cholinergic neurons of the nucleus basalis of Meynert (nbM).

Material and methods

Male Wistar rats 3.5 months old at time of surgery, were employed.

Lesion

Animals were anaesthetized with Equithesin (Jansen Lab., 2.5 ml/Kg intraperitonally) and placed in a stereotaxic apparatus (David-Kopf). Incisive teeth were fixed in a position 3.3 mm under the horizontal plane passing through the auditive fixing bars (Paxinos and Watson, 1982). With the (0.0) point corresponding to the bregma, the coordinates of the injection were: −1.3 mm posterior to the bregma; −2.5 mm lateral to the midplane; and −6.7 mm vertical to the duramater. The quisqualic acid neurotoxin was dissolved in 50 mM phosphate buffer pH 7.4 and 0.5 µl was microinjected following this schedule: placement of the Hamilton syringe (of 0.5 mm-diameter needle) at the chosen coordinates for 3 minutes, infusion of 0.1 µl every 30 seconds and maintenance of the syringe in place for 3 minutes before taking it out. Two series of unilateral (right hemisphere) nbM-lesions were performed by injection of solutions of 60 or 100 mM of quisqualic acid. Thus, two nbM lesioned groups of rats were utilized, one consisting of 30 nmols of quisqualic nbM-lesioned animals (30 Q) and the other consisting of 50 nmols of quisqualic nbM-lesioned animals (50 Q). Sham-operated control animals were injected in a similar manner with 0.5 µl of vehicle.

Treatment

One half of each group of lesioned animals was treated with Pyritinol dihydrochloride monohydrate (E. Merck, Darmstadt) for 21 or 45 days starting 24 h after lesioning. The

drug dose (200 mg/kg body weight) was administered every morning by gavage, after being dissolved in water at a concentration of 20 mg/ml.

Series of brains

Animals: sham operated, lesioned, lesioned and treated, were all sacrificed 21 or 45 days after surgery. Two series of brains from every group were obtained, fresh (for biochemical and autoradiographic studies) and fixed (for morphological and histochemical studies).

Preparation of samples for biochemical and autoradiographic studies

The fresh brains were divided into two groups. The brains of the first group were transversally cut 0.2 mm frontal to the injection point level. From the back of the anterior block, two 2.0 mm thick slices were obtained. From the first slice, two cortical bilateral samples, right and left, were dissected from the cingulate cortex to the rhynal fissure. These samples were homogenized (1/20, w/v) with 0.1 M phosphate buffer containing 10 mM EDTA and 0.5% Triton X-100. From the second slice, two similar samples were obtained and homogeneized in 0.32 M sucrose. From the posterior block, quickly frozen on dry ice, serial tissue sections (10 microns thick) were obtained and mounted on gelatin-coated glass slides at −20°C. The majority of these slides were kept at −20°C for autoradiography and the rest, after 4% formaldehyde fixation, utilized for morphological purposes (cresyl violet staining) and for the histochemical demonstration of the acetylcholinesterase (AChE) (Koelle and Friedenwaldt, 1949) to determine the location and the extent of the lesion. The brains of the second group were processed in a similar way but in this case, the anterior block (up to the septal level) was utilized for autoradiographic studies and the posterior block for biochemical studies. Cortical samples were dissected after discarding the injection-damaged area (a slice of approximately 0.5 mm). From this latter slice, two (right and left) mbM samples were also obtained by micropunch and homogenized in phosphate buffer after combining all the right and all the left sides.

Neurochemical determinations

In the phosphate buffer homogenates, the following determinations were performed: acetylcholinesterase (AChE) (Ellmans et al., 1961), in duplicate, and choline acetyltransferase (ChAT) (Fonnum, 1975), in triplicate, with Acetyl Coenzyme A [acetyl-^{3}H] (NEN; 4.7 Ci/mmol) as a substrate.

From the sucrose homogenates, crude mitochondrial P_2 subcellular fractions were obtained by centrifugation (Gray and Whittaker, 1962) and utilized for high affinity choline uptake (HACU) determination using choline methyl chloride [methyl-^{3}H] (NEN; 64.3 Ci/mmol) (Simon et al., 1976; Pedata et al., 1982).

Protein content was determined by the method of Lowry et al. (1951).

Autoradiographic studies

In unfixed slides, M_1 and M_2 receptors were identified using 1 nM scopolamine methyl chloride [N-methyl ^{3}H] (New England nuclear Prod./Du Pont, 73.8 Ci/mmol) to label them, and 300 nM pirenzepine and 100 μM carbachol to block subtypes and differentiate the two classes of receptors. Incubation time was 1 h, temperature 25 °C and 10 μM atropine the total displacer to produce blanks (Cortés et al., 1984). Ultrofilm (LKB) was employed to produce autoradiograms. To obtain the best results, exposure time was 14 days at 4°C with a 100% atropine displacement (Fig. 3). Autoradiograms were quantified using an Image Analyzer based on the Image Pro System (Media Cybernetics Inc., USA). Amersham ^{3}H micro-scales were used to calibrate the system.

Histological methods

The fixed brains, obtained by intracardial perfusion of 4% p-formaldehyde in phosphate-buffered saline (PBS) and 2 h of post-fixation in the same mixture, were washed/-cryoprotected by immersion in 30% sucrose and divided into two groups. Blocks of suitable shape and size were prepared to obtain serial coronal cuts from the anterior commisure up to the end of the caudate/putamen. The brains of the first group were cut in a cryotome to produce 20 μm serial coronal histological sections mounted on gelatin coated glass slides, which were evenly distributed into 4 series, taking every fourth section for each series. One series was stained with cresyl violet or toluidine blue (to study lesional changes and gliosis) and three series were employed to illustrate AChE with the Koelle and Friedenwaldt method (1949). The incubation times of these last three series were, approximately, 4; 3 and 2.5 hours, to demonstrate positive nbM-neurons and cortical fibres, nbM-neurons only, and hypertrophic (AChE hyperactive) nbM neurons, respectively. Brains of the second group were cut with a Leitz sledge microtome to prepare 40 μm coronal sections which were then processed for ChAT immunocytochemistry following the unlabelled antibody peroxidase-antiperoxidase (PAP) method (Sternberger, 1974). Spontaneous-floating sections were successively incubated in a medium with 1:50 rat monoclonal antibody (Boehringer Mannhein), in a solution 1:10 rabbit anti-rat IgG (Miles) and in a medium with PAP monoclonal antibody (Martinez-Murillo et al., 1990). The immunoreaction was revealed by incubating the sections in 0.06% 3,3′-diaminobenzidine and H_2O_2. AChE and ChAT positive cells in right and left nbM were studied and counted. AChE-positive fibers in cortical layer II, 3.5 mm lateral to the mid plane, per 100 square microns, were calculated using the above-mentioned Image Analyzer.

Statistics

In all cases, the value obtained in the right injected side was expressed as a percentage of the one found in the left non-injected side. Student's t-test was employed to compare groups.

Results

Cortical cholinergic parameters after nbM lesioning

Sham-operated animals did not present any change in their cortical cholinergic parameters at 21 or 45 days after nbM injection when the left sides were compared with the right sides of these animals and the left sides of unoperated animals. Pyritinol-treated sham-operated animals did not present any change in their cortical cholinergic parameters at 21 or 45 days after nbM injection when the left sides were compared with the right sides of these animals, and also the left sides of sham-operated and untreated animals. Therefore, the left cerebral hemisphere (contralateral to the lesion) was considered to be a suitable control in each animal (100% value) and the percentage of difference between right (lesioned) and left (unlesioned) was used to quantify the lesion.

The cortical cholinergic changes following nbM lesioning were dependent on the toxin concentration, and the evolution of the deficits related to both the neurotoxin concentration and the nootropic treatment. The cholinergic deficits were similar from +4 mm (frontal cortex) to −4 mm (parietal cortex) about the lesional plane.

Table 1. Average values (%; mean ± SD) of cholinergic parameters in 30 nmol quisqualic acid-(30 Q) and 50 nmol quisqualic acid(50 Q)-nbM-lesioned rats, with (PT) or without (NT) Pyritinol treatment, on the 21st and 45th day post-lesion. Data are expressed as a percentage of the contralateral non-lesioned side in each case. Absolute values (100% values) in contralateral non-lesioned frontoparietal cortex were: Acetylcholinesterase (AChE) = 68.2 ± 5.8 nmols ACh hydrolysed/mg protein per minute; Cholinacetyltransferase (ChAT) = 52.9 ± 4.5 nmols ACh formed/mg protein per hour; High affinity choline uptake (HACU) = 1.01 ± 0.09 *pmols choline/mg protein per minute*; Muscarinic type 1 receptor (M_1) = 952 ± 48 fmols/mg protein; Muscarinic type 2 receptor (M_2) = 339 ± 32 fmols/mg protein. (*n*) number of animals; [Σ6] = six samples from six animals processed all together; * = $p < 0.02$; ** = $p < 0.003$; *** = $p < 0.00005$ (in the statistical comparison PT/NT)

	30 Q				50 Q			
	21 days		45 days		21 days		45 days	
	NT	PT	NT	PT	NT	PT	NT	PT
Frontoparietal cortex								
AChE	75.1 ± 12.3	100.1 ± 19.5	92.3 ± 16.2	88.5 ± 10.5	52.8 ± 13.2	68.3 ± 17.1	54.7 ± 14.7	66.1 ± 18.7
(n)	(15)	(13)**	(15)	(13)	(12)	(11)*	(12)	(13)
ChAT	60.2 ± 11.3	86.1 ± 15.4	83.5 ± 17.5	86.2 ± 14.3	53.1 ± 17.7	56.6 ± 15.1	57.2 ± 13.8	66.1 ± 17.7
(n)	(15)	(13)***	(15)	(13)	(12)	(11)	(12)	(13)
HACU	64.3 ± 12.3	84.8 ± 13.2	80.3 ± 14.4	91.9 ± 10.9	58.0 ± 12.2	62.2 ± 13.8	60.7 ± 15.5	69.9 ± 17.3
(n)	(9)	(7)***	(9)	(7)	(7)	(6)	(6)	(7)
M_1	93.2 ± 3.2	95.2 ± 6.2	92.2 ± 6.2	92.0 ± 8.3	91.3 ± 4.9	95.2 ± 8.2	89.2 ± 5.6	94.2 ± 8.2
(n)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)
M2	86.3 ± 8.6	89.3 ± 6.3	86.3 ± 8.0	86.3 ± 6.4	84.2 ± 8.8	86.2 ± 6.0	80.5 ± 5.8	81.2 ± 8.3
(n)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)
Nucleus basalis [Σ6]								
AChE	26.0	28.6	31.0	25.3	19.5	18.3	22.5	21.6
ChAT	26.7	22.7	28.2	27.9	22.8	19.5	23.3	25.7

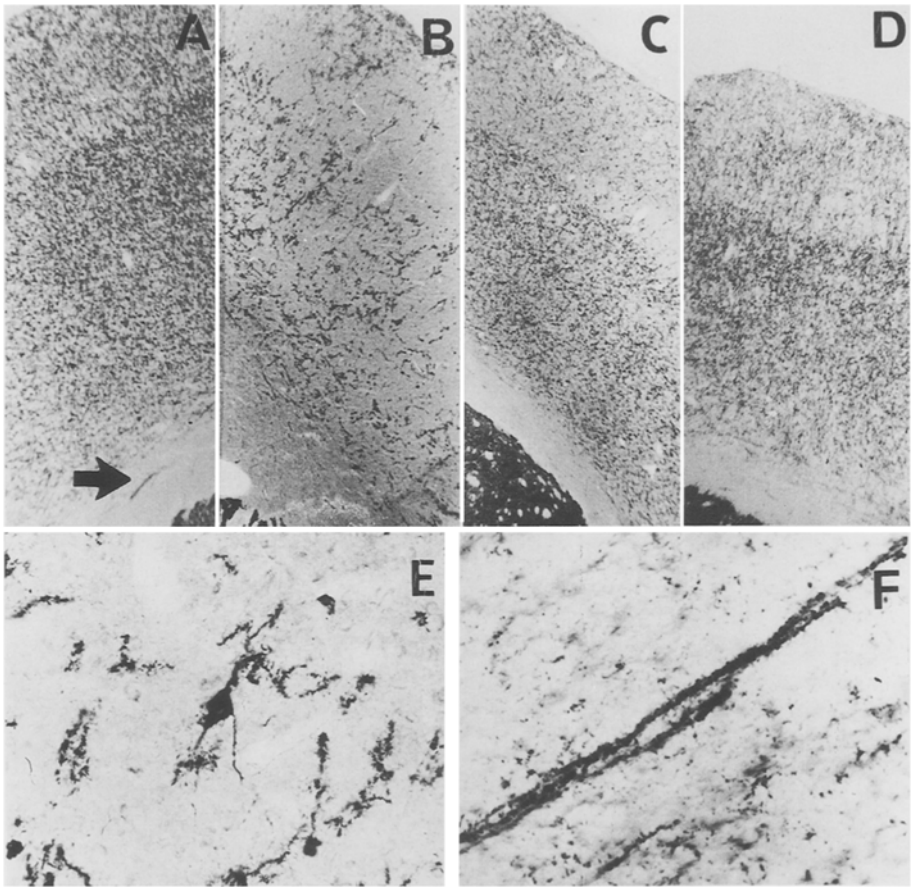

Fig. 1. Histochemical AChE positive network in the frontoparietal neocortex of several cases of Pyritinol treated and non treated animals after quisqualic acid lesioning. **A** Dense AChE network in the left contralateral non-lesioned side (control). **B** Isolated fibers and neurons with AChE reactivity in the right ipsilateral side to a nbM injection with 50 nmols of quisqualic acid, 45 days after lesioning and with 45 days of Pyritinol treatment. **C** A partial loss of AChE positive fibers ipsilateral to a nbM injection with 30 nmols of quisqualic acid, 21 days after lesioning and without Pyritinol treatment. **D** Dense AChE network ipsilateral to a nbM injection of 30 nmols of quisqualic acid, 21 days after lesioning and with a simultaneous Pyritinol treatment. **E** Intense cortical AChE-positive neuron and **F** hypertrophic fibers (thicker and more heavily stained than the normal) in the right ipsilateral side to a nbM injection with 50 nmols of quisqualic acid, 45 days after lesioning and with 45 days of Pyritinol treatment. (A/C = 30×; E,F = 300×). → = hypertrophic fiber in non-lesioned side

In the biochemical study of lesioned rats, AChE, ChAT and HACU underwent a significant, and dose dependent, decrease after nbM lesioning (Table 1). The Pyritinol treated groups varied in response depending on the severity of the lesion. In the 30 Q model a recovery of AChE, ChAT and HACU average values was observed after 21 days of treatment; after 45 days this showed no statistically significant difference from un-treated animals. In the 50 Q model, only AChE after 45 days exhibited any recovery.

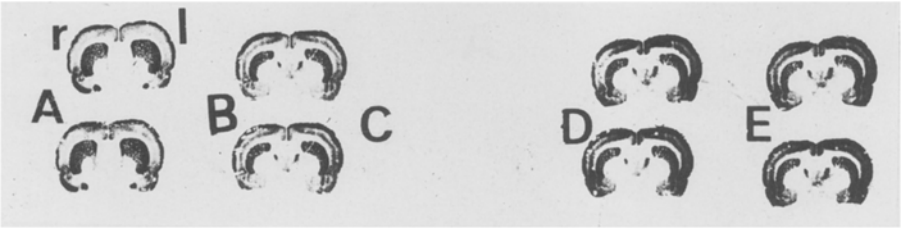

Fig. 2. Direct reproduction of autoradiograms from a Pyritinol-treated animal in which the densitometric measurements of the frontoparietal neocortex displayed a decrease of M_1 receptors by 9.6% and a decrease of M_2 receptors by 22.1% ipsilateral to a nbM-lesion with 50 nmols of quisqualic acid (40.6% decrease of AChE and 38.8% decrease of ChAT were determined in the cortex). All slides were incubated in 1 nM ^{3}H-N-methyl-scopolamine; in **D** and **E** no displacer was added; in **C** 10 μM atropine was included (blank); in **B** 300 nM pirenzepine and in **A** 100 μM carbachol were added to distinguish M_1 and M_2 receptors (see text). (*l* left side; *r* right side)

In the histochemical study, variations of both AChE positive fibers and AChE or ChAT positive neurons were observed. Lesions provoked a diminution of AChE positive fibres in all the layers of the ipsilateral fronto-parietal cortex, especially those in which the cholinergic network was more complex (Fig. 1). At 21 days this deficit could be estimated (by determining the number of AChE positive ascending fibers in cortical layer II) at 29 ± 5% for 30 Q and 59 ± 9% for 50 Q. At this time, all the Pyritinol-treated and non-treated animals in the 50 Q group had the same histochemical appearance, but the treated rats of the 30 Q group showed a denser AChE network, similar, in various sections, to the contralateral unlesioned side. At 45 days, both treated and non-treated animals with a lesion of 50 nmol maintained the deficits of positive fibers, but all the animals with 30 nmol lesions had a fairly normal AChE network. It was noted that hypertrophic fibers (Fig. 2) could be observed at 21 and 45 days in non-treated animals as well as in treated animals, ipsilateral to the lesion (Fig. 1). A higher number of these fibers were observed in the pyritinol-treated 30 Q animals sacrificed at 21 days. In the contralateral non-lesioned side, some fibers of this type were observed in treated and in untreated, 30 Q and 50 Q animals (Fig. 2). The number of positive AChE cortical neurons in either hemisphere (Fig. 1), randomly distributed and of different shapes and sizes, increased at 21 and 45 days after lesion (from 18–39/section to 22–63/section in the case of 30 Q animals at 45 days, right side). The number of these hyperactive cells in treated animals did not increase in comparison with the untreated animals. ChAT positive neurons in the frontoparietal neocortex were also observed in both hemispheres in lesioned animals and lesioned + Pyritinol treated animals. The number of these cells was very irregular throughout the cortex, but higher in the lesioned side (0–12/section in the left side and 0–18/section in the right side). In 40 cuts taken at random from every group (lesioned and lesioned + treated animals), there was no relationship between the number of ChAT positive cells and the treatment in the cortex ipsilateral to the lesion.

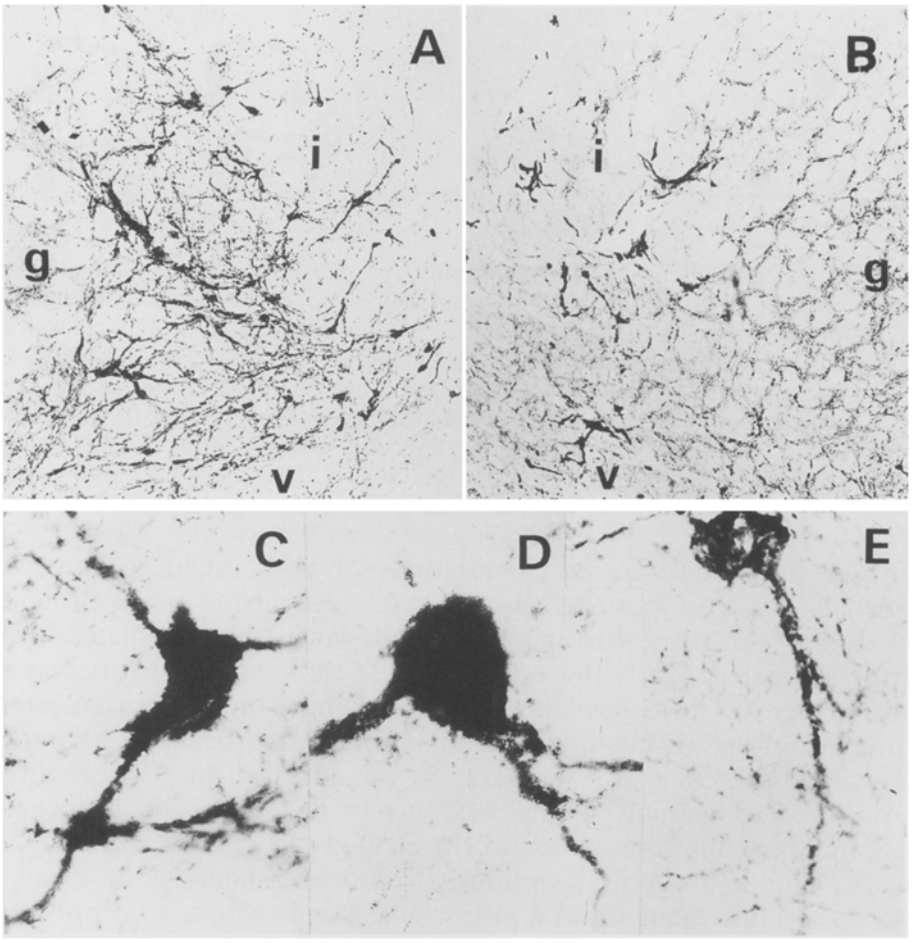

Fig. 3. AChE in nbM neurons and fibers in the left non-lesioned side (**A**) and in the right lesioned side (**B**) in a rat 45 days after a 50 Q lesion. Normal neurons and a dense AChE network is displayed in A_1 and a general loss of positive neurons can be observed in B. In this image, the remaining neurons and fibers are of hypertrophic or dysplasic appearance. In C, D and E, the main types of large AChE-positive cells considered in the text are shown: **C** normal, **D** hypertrophic, **E** dysplasic. *g* globus pallidus; *i* internal capsule; *v* ventral pallidum

The autoradiographic study (Fig. 2) presented a small deficit of muscarinic receptors after lesioning, the results being unchanged by the Pyritinol treatment (Table 1). M_2 receptor subtype displayed a greater decrease in both 50 nmol lesioned and 50 nmol lesioned + treated animals.

nbM cholinergic alterations

No changes were observed in sham-operated animals, treated or non treated, but the biochemical deficits at the injection site (Table 1) in lesioned animals were substantial.

Histochemically, alterations were observed in the nbM and neighbouring areas. In an area of about 0.2 mm diameter around the lesion point, no

cholinergic neurons were observed after 30 or 50 nmol lesions, but the most important dose-dependent variations were observed in a region of the nbM corresponding to the forebrain delimited by the planes −0.8 mm anteroposterior and −1.8 mm anteroposterior of Paxinos and Watson (1982).

In this region, it was possible to observe many surviving cholinergic (AChE and ChAT positive) cells in 30 Q rats, 21 and 45 days after surgery, both in non-treated and treated rats. AChE intensity of these cells seemed to be higher in treated animals, particularly in the 21 day lesioned animals. The morphology of these cells after AChE staining was variable (Fig. 3): a) large cells similar to those in the non-lesioned side (large, stellate and homogeneously stained); b) hypertrophic/strongly AChE positive stellate cells; c) large non-homogeneously stained (dysplasic) cells and d) small cells similar to the small cholinergic cells in the non-lesioned side. a), b) and c) types were also observed after ChAT immunostaining. The number of each type varied from section to section in each animal but a few cells of the first normal type were observed at 21 days, when most of the surviving neurons were hypertrophic or dysplasic, and, conversely, the most cells at 45 days were of normal appearance. At 45 days the ChAT-positive cells were decreased by 36 ± 6% in the non-treated group and by 30 ± 7% in the Pyritinol treated group.

In 50 Q rats, fewer AChE- and ChAT-positive neurons were observed in the −0.8/−1.8 mm nbM lesioned area. AChE-hypertrophic cells were rare in all cases. At 45 days, the ChAT decrease was 64 ± 12% and 58 ± 8% in non treated and Pyritinol treated groups.

AChE- and ChAT-positive neurons, most of them hypertrophic, were observed in neighbouring areas of the nbM. In 30 nMol quisqualic acid lesioned animals, more of these neurons could be seen in the upper part of the internal capsule, in the infrapallidal area, in the pallidum and the anterior part of the nbM close to the anterior commisure. In the posterior part of the nbM along the optic tract, both the number and histochemical intensity of the neurons were similar in 30 nmol and 50 nmol lesioned animals, both treated and non-treated.

A general atrophy of the nbM region was observed in the lesioned side of treated and untreated animals. Gliosis (particularly at 45 days), cell debris, the injection tract and espheroid bodies were indicators of the site of the injection in perfused and non perfused brains.

Discussion

In the two groups of quisqualic-nbM-lesioned rats utilized in the study, cortical cholinergic deficits, except in M_1 receptors, were observed 21 days after surgery in the lesioned side; these included deficits in the activities of AChE, ChAT and HACU; the AChE histochemical network and the autoradiographic density of M_2 receptors. The most obvious differences at this time between the 30 Q and the 50 Q animals related to dose. These general characteristics were in agreement with the results described by other authors (De Micheli and Soncrant, 1992; El Defrawy et al., 1985; Gardiner

et al., 1987; Gaykema et al., 1992; Gower, 1986; Haroutunian et al., 1986b; Mash et al., 1985; Riekkinen et al., 1991; Robbins et al., 1989; Santos-Benito et al., 1988; Vige and Briley, 1989; Wenk and Olton, 1984). For some years lesions of nbM have been considered valuable models for AD (Gower, 1986; Haroutunian et al., 1986b; Smith, 1988; Toledano, 1992). Although several authors have questioned this (Fibiger, 1991), there is no doubt of their value in studying the cortical cholinergic innervation arising from the basal forebrain (Perry et al., 1991; Ridley et al., 1991; Riekkinen et al., 1991; Sarter et al., 1991).

Spontaneous recovery of the deficits at 45 days has been observed in the 30 Q group, while maintenance of the deficits at 45 days has been already described in the 50 Q group. Spontaneous recovery of the deficits has previously been reported following various lengths of time, notably in unilateral lesions (Casamenti et al., 1988; Dunnet et al., 1987; De Micheli and Soncrant, 1992; Gardiner et al., 1987; Pedata et al., 1982; Santos-Benito et al., 1988; Vige and Briley, 1989; Wenk and Olton, 1984).

In the two examples used, an injury of the cholinergic neurons in the central region of the nbM was demonstrated by profound decreases in AChE and ChAT activities, and a loss of cholinergic neurons in the area of injection. But the characteristics of the lesion were very different in the two groups. The number of surviving neurons (AChE and ChAT positive neurons) in the 30 Q rats were far superior to the number in the 50 Q animals. Moreover, in 30 Q rats, many cholinergic cells seemed to be hypertrophic on the 21st day after surgery. If the number of ChAT neurons decreased on the 45th day after surgery and cholinergic parameters recovered up to the normal contralateral levels, the possibility could exist that hyperactivity of the surviving neurons was occurring. Reinnervation of cortical cells (neurons which were the main targets of the irreversible damaged nbM neurons) by axonal sprouting from the remaining cells could also be involved in the process of recovery. This has been discussed in several experimental models (De Micheli and Soncrant, 1992; Gage et al., 1983) and needs additional study at the electron microscopic level to be confirmed. It can be concluded that both the existence of surviving neurons in the nbM and their responses to injury are of prime importance in the development of cortical cholinergic deficits. Important too is the response to the injury of several subsets of cholinergic neurons, cholinergic neurons in the ipsilateral neighbouring areas of the nbM functionally related to the damaged cells (Alheid and Heimer, 1988; Toledano, 1992), contralateral nbM neurons (Pearson et al., 1986) and cortical cholinergic neurons (Levey et al., 1984; Toledano, 1992), should be taken into account.

Changes in surviving nbM neurons after lesioning have been observed (Gaykema et al., 1992), but their actual significance has not been well established.

The dispersion of nbM-cholinergic cells (Alheid and Heimer, 1988; Toledano, 1992; Zilles et al., 1991) can explain some features of the results (high standard deviation or individual variations (Table 1); high differences among sections taken at random; etc).

If spontaneous recovery of the deficits in 30 Q animals could be related to hyperactivity or plasticity of the cholinergic neurons (Pedata et al., 1982) (as indicated by the hypertrophy and the AChE/ChAT/HACU hyperactivities observed in the results), the earlier recovery induced by Pyritinol could be produced through an enhancement of the activities of the surviving cholinergic nbM neurons; higher cortical activities and more hypertrophic cortical fibers and nbM neurons were observed at 21 days in treated rats in 30 Q.

Pyritinol has been shown to enhance decreased cholinergic function in animals with cholinergic impairments (Marston et al., 1987) and also has therapeutical effects in diseases with cholinergic disturbances (Herrmann et al., 1986, 1988; Knezevic et al., 1990; Maurer, 1989; Seyfried, 1989). These effects could thus relate to increased activity of the cholinergic nbM neurons on the basis of the results here reported. It is interesting to note that Pyritinol did not enhance cholinergic parameters in sham-operated animals, as occurred with normal young rats described by Marston et al. (1987). In AD, regenerative and degenerative changes always co-exist in the nucleus basalis of Meynert (Iraizoz et al., 1991), therefore it is possible to assume that a number of these neurons could respond to Pyritinol. Although a number of cholinergic neurons always remain in the basal forebrain (Chui et al., 1984), earlier diagnosis and treatment may well improve cholinergic activity with a greater potential for therapeutical benefit.

Neurotrophic factors such a NGF or gangliosides (Hefti et al., 1984, 1989; Maysinger et al., 1989, 1992; Snider and Johnson, 1989) have promoted the recovery of injured cholinergic neurons of the basal forebrain (Fisher and Björklund, 1991; Haroutunian et al., 1986a; Hefti et al., 1984; Maysinger et al., 1989, 1992), prevented the damage induced by neurotoxins (Hefti, 1986; Koliatsos et al., 1990) and stimulated cholinergic activities (Williams, 1991). However, no neurotrophic factor has been easily accepted for therapeutic assessment in AD or other cholinergic-related CNS syndromes (only one case report is now available, Olson et al., 1992). Pyritinol and the other nootropics demonstrating activating properties on cholinergic recovery could be considered as external neurotrophic factors similar to the above-mentioned substances. This could be important because treatment with neurotrophic factors is now strongly stressed (Hefti et al., 1989; Morgan, 1989; Snider and Johnson, 1989) since the above mentioned "cholinergic" therapies have been of limited use (Bartus et al., 1985; Palacios and Spiegel, 1986; Fibiger, 1991).

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