

The Cerebrovascular Effects of Physostigmine Are Not Mediated through the Substantia Innominata

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This study sought to determine whether the cortical cholinergic projections from Meynert's nucleus are actually the target of the cholinesterase inhibitor physostigmine, which presents the ability to increase cortical blood flow. To this aim, the multiregional cerebrovascular effects of physostigmine in rats with and without lesion of the substantia innominata (SI), the equivalent of Meynert's nucleus of primates, were investigated. Unilateral SI lesions were made using ibotenic acid in three groups of rats. Four to 11 days later, the cortical choline acetyltransferase (ChAT) activity was measured in one group to assess the efficacy of the lesion. In the two other groups, the regional cerebral blood flow was measured using the [¹⁴C]iodoantipyrine technique, under physostigmine (0.2 mg/kg/h iv) or control conditions. SI lesion induced 27–59% fall in cortical ChAT activity in the ipsilateral hemisphere with the frontal area most affected. Despite these large biochemical differences, the lesion had little cerebrovascular effects. Side-to-side blood flow differences did not exceed 11% and did not strictly overlap the ChAT depletion. Physostigmine increased flow (38–66%) in all cortical areas, with no frontal predominance. Despite these considerable vasodilations, there were no significant differences between the lesioned and the intact hemisphere, nor any significant interaction between physostigmine and SI lesion. Thus, physostigmine does not actually activate the SI neuron terminals. This result suggests that cholinesterase inhibitors cannot be used as presynaptic markers of the cholinergic activity of this nucleus and casts doubts on their specificity as enhancement therapeutic agents in Alzheimer's disease.

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INTRODUCTION

The "cholinergic hypothesis" of Alzheimer's disease is based on the low choline acetyltransferase (ChAT) activity (enzyme of acetylcholine synthesis) found in the brains of these patients (for references, see 11, 45). It assumes that the cognitive impairments of this dis-

ease are mainly due to the cholinergic deficit caused by degeneration of the nucleus basalis of Meynert, which is the main source of cholinergic innervation of the neocortex (14, 34, 40). By analogy with the replacement therapy applied to Parkinson's disease, this hypothesis has provided a rational basis for a therapeutic strategy aimed at increasing the concentrations of acetylcholine (Ach) in the brain by inhibiting the activity of cholinesterase (enzyme of Ach catabolism). Cholinesterase inhibitors are assumed to maintain the endogenous Ach concentration wherever it is released in the brain, including particularly, the cortical projection areas of the central cholinergic systems.

Physostigmine (eserine) has been widely used to that purpose, but the results have been less beneficial than expected and have become a matter of debate (15, 36, 45). Thus, the practical usefulness of cholinergic agents for treating Alzheimer's disease (AD) was further in question. Indeed, there is as yet no direct evidence that this therapy intervenes in the disturbed cholinergic function of the cortically projecting neurons, to specifically compensate for a functional deficit of Meynert's nucleus.

Animal experimentations have shown that physostigmine increases cerebral blood flow (CBF) (46) and have demonstrated a functional link between the substantia innominata (SI) and the cortical circulation. Stimulation of the SI induces considerable cortical vasodilation in the rat, whether conscious or anesthetized (2, 6, 30), which is strongly potentiated by physostigmine (6, 31) and reduced by scopolamine (7), indicating that cholinergic mediation is clearly involved in the functioning of the innominatocortical system. These data are the basis of the present experimental design which thus postulates that CBF provides a convenient biological measure of the pharmacodynamic effects of physostigmine. We sought to answer the question of whether the cholinergic system of Meynert's nucleus is actually the target of physostigmine. To this aim we have used the experimental model of unilateral lesion of the SI to study in the conscious rat the cerebrovascular effect of iv infused

physostigmine in the lesioned and intact hemispheres compared to that in the untreated rat.

Part of this study has been published in abstract form (28).

MATERIALS AND METHODS

Thirty-two male Sprague-Dawley rats weighing 280–330 g were used in a two-step surgical procedure: lesioning of the SI, followed by ChAT activity or CBF measurements 4 to 11 days later (6.4 ± 2.3 means \pm SD).

Lesioning of the SI

In the rat, this region includes the nucleus basalis magnocellularis, the equivalent of Meynert's nucleus in primates, where most of the cholinergic cortically projecting neurons are located (14, 40).

Rats were anesthetized with chloral hydrate (400 mg/kg, ip, in an 8% solution) and placed in a stereotaxic frame (D. Kopf Instruments 1400) and their skulls were exposed and drilled on the left side. A stainless steel cannula (the shaft of a 0.3-mm o.d. hypodermic needle) was then inserted into the SI (1/100 mm micropositioner DKI 1760/61) so that the tip was located at the following coordinates (mm): interaural axis, anterior, 7.2; lateral 2.9; horizontal, +2.6 (incisor bar set at -5.0). One microliter of ibotenic acid (Sigma I 0382, 10 μ g in sodium hydroxide-buffered saline) was slowly infused (200 nl/min) through the cannula connected to a 10- μ l syringe (Hamilton 1801 NE, Switzerland) driven by a microperfusor pump (Carnegie Medicine CMA 100, Sweden). Particular care was taken to avoid diffusion of the neurotoxin along the intracerebral track of the cannula. The cannula was left in place for 1 min after completion of the 5-min infusion. It was then withdrawn by 0.5 mm and left in place for 2 additional min before being slowly removed (1/100 micromanipulator). The outside of the cannula was carefully cleaned of ibotenic acid before each use.

ChAT Activity

A group of 14 lesioned rats was sacrificed under deep halothane anesthesia for both ChAT activity measurement and histological examination of the lesion. Tissue samples (30–50 mg) from the neocortex, the SI projection areas, and from the cerebellar hemisphere, where the SI does not project, were frozen on dry ice and stored at -70°C until enzymatic analysis.

ChAT activity was measured by the procedure of Fonnum (17). Tissues were sonicated for 30 s in EDTA buffer (pH 7.4) containing 0.5% Triton X-100, and 10- μ l aliquots of supernatant were added to 10 μ l of assay mixture containing 0.1 mM [^{14}C]acetyl-CoA, 0.3 mM S-acetyl-CoA, 0.6 M NaCl, 0.1 M sodium phosphate buffer (pH 7.4), 16 mM choline chloride, 0.2 mM eserine

salicylate. The mixtures were incubated for 15 min at 37°C . The [^{14}C]Ach formed was extracted with sodium tetraphenylboron into a scintillation cocktail. Each assay was performed in triplicate. The homogenate protein content was determined by the method of Lowry *et al.* (39).

CBF

The CBF technique is based on the Fick principle, using [^{14}C]iodoantipyrine as a diffusible tracer (44), combined with brain tissue sampling. The CBF measurement started with infusion (1.2 ml/min) of a solution containing 40 μ Ci tracer (4-iodo-[*N*-methyl- ^{14}C]antipyrine, Commissariat à l'Énergie Atomique, sp act 54 mCi/mmol) for a period of 40 s. Simultaneously, a total of 14 arterial blood samples (about 45 μ l) were collected in microtubes, one every 3 s. At the end of infusion the heart was arrested with a bolus of 1 ml of 6% pentobarbital (w/v), producing a precipitous fall in arterial blood pressure, and the rat was decapitated. The brain was rapidly removed and dissected into 11 regions bilaterally on a cool metal plate. These procedures were performed as fast as possible to minimize postmortem tracer diffusion within the brain tissue compartments. The brain samples were placed in pre-weighed scintillation vials and the vials were reweighed.

The arterial blood samples were centrifuged and 20- μ l plasma samples were placed in scintillation vials. The uniform tracer partition between plasma and red cells was verified. A mixture of tissue solubilizer (Solute, Baker) and propanol-2 (4 V, 1 V) was added to the vials containing blood and brain samples. Brain samples were digested for 4 h at 50°C with gentle shaking. All vials were filled with 5 ml scintillation cocktail (Lipofluor, Baker) and their radioactivity was counted (SL 3050 Intertechnique).

The absolute blood flow in each brain region was calculated from the tissue sample radioactivity and time-contamination curve of arterial blood samples, using the integrated equation of Kety. The brain-blood partition coefficient used for iodoantipyrine was 0.8 (44).

Surgical Procedure

Rats were anesthetized with 1–1.5% halothane, femoral veins were catheterized for infusion of physostigmine and tracer, and femoral arteries for continuous arterial pressure recording and arterial blood sampling. The skin incisions were covered with lidocaine hydrochloride (2% Xylocaine gel) and carefully sutured. The rats were then comfortably installed in a hammock with the limbs attached underneath without excessive constraint. Head motility was limited by a device gently maintaining the neck, in order to administer various respiratory gas mixtures (hypercapnia) as required for

other experiments (7). The rat was then allowed to recover from anesthesia.

Experimental Protocol

CBF was measured 2 h after the end of halothane anesthesia in the conscious, locally anesthetized rats. Arterial blood pressure (mean and direct) and heart rate were continuously recorded (Gould 3400) throughout the experiment. The colon temperature was maintained at its usual initial value ($37^{\circ}5$) using a temperature-controlled heating blanket (Homeothermic system, Harvard).

An initial dose ($25 \mu\text{g}/\text{kg}$) of physostigmine (eserine, Sigma E 8625) was given iv 20–30 min before CBF measurement, followed by continuous infusion ($0.2 \text{ mg}/\text{kg} \cdot \text{h}$, Precidor perfusor, Infors, Switzerland). This protocol induces significant, stable effects on CBF and limited side effects (hypertension) as observed using the continuous mass spectrometry technique (31). The control group was perfused with saline at the same rate ($1.2 \text{ ml}/\text{h}$).

Arterial blood gases and pH were measured (Corning 178 analyzer) before infusion and just before CBF measurement.

Histological Examination of SI Lesion

The lesion sites were examined and located in seven rat brains used for ChAT activity measurement. The remainder of the brain after cortical sampling was frozen at -40°C in a mixture of isopentane–freon and mounted on a chuck with embedding medium (M1 Lipshaw). Serial forebrain sections ($20 \mu\text{m}$) were cut at -20°C in a cryostat (Bright 5030). The lesioned areas were visualized on cresyl violet-stained sections. High-power cytoarchitectonic examination showed a loss of neuronal perikarya associated with proliferation of nonneuronal cells. At low power, the needle tip was identified by the dense darkly staining nonneuronal cells around the lower part of the track and recorded on standardized brain sections as previously described (31).

Experimental Groups and Statistical Analysis

The rats from each lesion series were assigned to one of the three experimental groups: one group for ChAT activity measurement and histological examination and two groups for CBF measurements. When the lesion placement was found to be inaccurate, the corresponding experiment was discarded. Likewise, rats with inflammation of the scalp, skull, or brain surface were excluded (two cases).

CBF was measured in two groups of nine conscious rats unilaterally lesioned in the SI. The CBF values of homotopic regions of the two hemispheres were com-

TABLE 1

Percentage Changes in Ipsilateral, Compared to Contralateral, Choline Acetyltransferase (ChAT) Activity, 4 to 11 Days Following Unilateral SI Lesion

Frontal cortex (14)	$-59.3 \pm 2.5\%^*$
Parietal cortex (14)	$-49.1 \pm 3.9\%^*$
Temporal cortex (14)	$-50.3 \pm 2.3\%^*$
Occipital cortex (14)	$-27.3 \pm 3.4\%^*$
Cerebellar hemisphere (7)	$-4.1 \pm 4.8\%$

Note. Values are means \pm SEM for the number of samples in parentheses.

* Significantly different from zero, $P < 0.001$, one-sample analysis *t* test.

pared using paired *t* tests. The significance of the effects of physostigmine was assessed by multiple comparisons (on four series of values) using two-factor ANOVA followed by Tukey's test for multiple-range analysis. This analysis also indicates the significance of the lesion effects and any interaction between lesion and physostigmine effects.

RESULTS

Efficiency of the SI Lesion

The efficiency of the SI lesion was assessed by the ChAT activity (Table 1) which decreased in the cortical areas ipsilateral to the lesion, by 27% in the occipital cortex and 59% in the frontal cortex. The greatest individual decrease was 74% (in the frontal and parietal cortex). This regional distribution is consistent with the known distribution of the neocortical SI projections (14, 34). The changes in ChAT activity in the cerebellar hemispheres (not significant) were in agreement with the absence of SI projections to this structure.

Behavior and Physiological Parameters

SI lesioning induced moderate, stereotyped, asymmetrical movements of the hindpaws (scratching) and body torsions or circling during recovery from chloral hydrate anesthesia. There were no long-term behavioral effects. The rats recovered their initial body weight within 4–6 days following the lesion.

Physostigmine significantly increased arterial blood pressure (by 16%, Table 2). This hypertension was progressive and was accompanied within 4–6 min by sustained stereotyped movements (chewing, vibrissae tremor, and sometimes gnawing), occasional abundant salivation, and moderate hyperventilation although changes in blood gases and pH were not significant.

TABLE 2
Physiological Parameters in SI-Lesioned Rats

	Control group	Physostigmine group
MABP (mm Hg)	115 ± 2.5	133 ± 4.2*
PaO ₂ (mm Hg)	96 ± 1.4	99 ± 1.6
PaCO ₂ (mm Hg)	39.5 ± 0.8	38.6 ± 0.9
Arterial pH	7.41 ± 0.018	7.40 ± 0.013

Note. Values are means ± SEM, $n = 9$ for both groups.

* Significantly different from control, $P < 0.01$, t test.

Effects of SI Lesion and Physostigmine on CBF

Unilateral SI lesion induced a small reduction in blood flow that was significant in only three cortical areas, frontal, parietal, and occipital, and never exceeded 11% (Fig. 1A). The dose of physostigmine used significantly increased the blood flow in all the brain regions investigated (unlesioned side), except the caudate nucleus (Fig. 1B). There were no significant side-to-side differences.

Figure 2 more directly shows the cerebrovascular responses to physostigmine on the lesioned and intact sides. Increases (15–66%) were greatest in the cortical areas (+38 to 66%) and the superior colliculus (+42%). The frontal cortex did not exhibit the largest response, and the temporal cortex was the least responsive cortical area. The distribution of this response was similar to the one of the side-to-side differences in flow due to SI lesion (Fig. 1A). None of the differences between the intact and lesioned sides was significant. Instead of the reduced responses on the lesioned side that might be

expected from the working hypothesis, there was a trend toward a greater effect on the lesioned side in four of the five most responsive structures. Two-factor ANOVA showed that the interaction between physostigmine and SI lesion was not significant in any of the regions studied.

DISCUSSION

Unilateral lesions of the SI induced marked decreases in the ipsilateral cortical ChAT activity in the conscious rat and slight decreases in blood flow. Physostigmine increased blood flow in most cerebral regions, especially the cortical areas in which the pattern of vasodilation did not correspond closely to the pattern of ChAT depletion. Above all, these responses were unaffected by the lesion.

Specificity of the Experimental Paradigm

In this model (conscious rat), physostigmine at the dose used is not totally devoid of side effects, such as hypertension, hyperventilation, or increased motility. Arterial blood pressure was significantly altered, but since it increased moderately and progressively while remaining well within the "autoregulated" range of blood pressure, it can be assumed not to affect CBF, especially in the conscious rat (31). Furthermore, our conclusions mainly based on side-to-side comparisons are largely independent of factors which bilaterally influence the cerebral circulation.

In this respect, the unilateral lesion paradigm may have two limitations. First, the few histologically demonstrated contralateral cortical projections from the SI

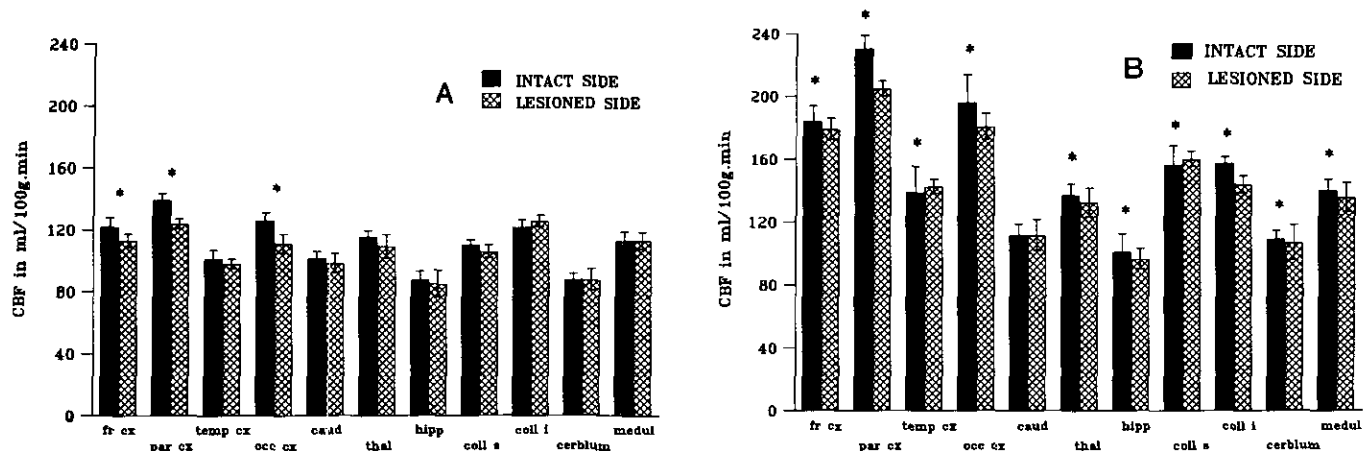


FIG. 1. Effect of unilateral SI lesion on cerebral blood flow (CBF) in (A) control rats and (B) physostigmine-treated rats (0.2 mg/kg/h iv). Values are means ± SEM in ml/100 g·min. *In A, significantly different from the intact side, $P < 0.05$, paired t test. *In B, significantly different from the control group (unlesioned side), $P < 0.05$, two-factor ANOVA plus Tukey's test. (The side-to-side differences are not significant, paired t test.) Abbreviations: Fr, frontal; par, parietal; temp, temporal; occ, occipital; cx, cortex; caud, caudate nucleus; thal, thalamus; hipp, hippocampus; coll, colliculus, s, superior, i, inferior; cerblum, cerebellum; medul, medulla (rostradorsal).

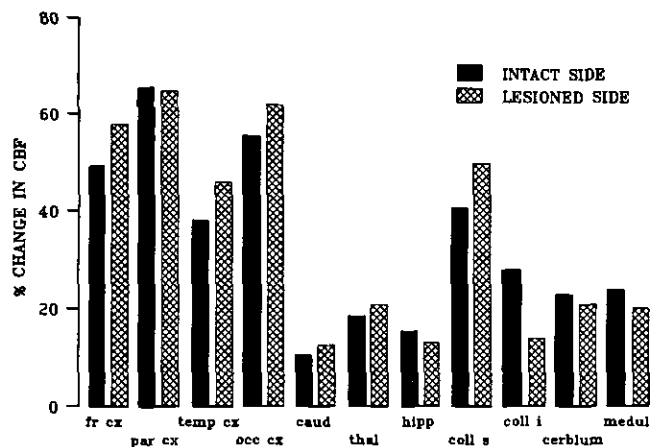


FIG. 2. Cerebrovascular responses to physostigmine on the lesioned and the intact side as expressed in percentage change in cerebral blood flow (CBF). Values were obtained by comparing Figs. 1A and 1B. None of the differences between the lesioned and the intact side are significant (two-factor ANOVA). Same abbreviations as used in Fig. 1.

(14, 34) will tend to limit the interhemispheric differences in ChAT activity and blood flow. However, comparisons of the cortical ChAT depletions following uni- or bilateral lesions have shown minor or negligible contralateral effects (12, 18, 48). Second, either the compensatory hypertrophy of the contralateral SI described by Pearson *et al.* (41), or denervation supersensitivity (33), would likewise result in reduced or inversed interhemispheric differences. Since these phenomena do not come into play before 15 days postlesion (41, 47), they will not be considered in the interpretation of our results.

Lesion Selectivity and Efficiency

There is evidence that ibotenic acid lesions have a limited selectivity in both their intracerebral location and their histological and pharmacological targets. Dunnett *et al.* (10) reported that the dorsal pallidum was often damaged following ibotenic acid microinjection into the SI. Our lesions showed no injury in the dorsal pallidum, and the location of the lesioned areas in the basal forebrain seemed less variable than sometimes reported. This improved selectivity may be due to the precautions employed: sharp cannula, 1/100 mm positioner, constant microperfusion (allowing progressive diffusion), and the use of the interaural axis as reference, instead of the potentially more variable bregma landmark.

Axon-sparing excitotoxins inevitably damage non-cholinergic neurons as well as cholinergic ones, so that some effects of ibotenate-induced SI lesions cannot be ascribed solely to the loss of cortically projecting neurons (9, 10, 15). However, the ChAT depletion induced in the cortex indicates a lesion efficiency rather higher

than usual (for comparison, see 10). The regional investigation shows a pattern of depletion with a frontal predominance, concordant with the known distribution of SI cortical projections (14, 34). The greatest individual decreases (74%) are consistent with early reports (12, 18, 48) and with the notion that approximately 70% of cortical ChAT originates in the basal forebrain, while the remaining 30% is due to the intrinsic cortical cholinergic neurons described by several authors (11, 23, 26). Thus, the cholinergic cortical deafferentation performed in this study was probably almost complete.

Cerebrovascular Consequences of SI Lesion

Despite a considerable neurochemical input, the cortically projecting cholinergic SI neurons produce only a mild tonic stimulation of the cortical circulation, as previously suggested (2). Their effects are comparable to those described in the rat by Gomi *et al.* (20), both in magnitude and distribution. This poor anatomofunctional correlation could be ascribed to a mostly phasic and modulatory influence of these projections, as shown by numerous studies (10, 15, 32), including enhancement of vigilance and coordination of somatosensory information processing in the cortex (for review, see 45). It may also explain the partial mismatch between the distribution of the cholinergic cortical projections from the basal forebrain as determined biochemically and the distribution of the functional cerebrovascular consequences of SI lesion or AD. In this last situation, the hypoperfusion is usually predominant in the parieto-temporal cortex (19, 24; and for reviews, see 29, 49).

Combined Effects of Physostigmine and SI Lesion

Our experimental design is mainly based on the findings that activation of the SI clearly induces cortical vasodilation (2, 6, 30, 38) potentiated by physostigmine (2, 7, 31). These results suggested an effect on Ach release from the cortical terminals of SI neurons, which has been confirmed by Kurosawa *et al.* (27). The inhibitory effects of scopolamine (a muscarinic receptor antagonist) reinforce this view (7). The vasodilatory effect of physostigmine on the cortex and its local bioavailability and appropriate dose are well established in the rat (46). This drug was therefore used in the present study to test cerebrovascular reactivity toward the cortically projecting SI neurons. If these neurons were the target of physostigmine, the cortical vasodilation would be less on the lesioned side, physostigmine acting on fewer functionally intact cholinergic neurons.

An extensive body of evidence converges to support this hypothesis. Physostigmine was shown, at first, to ameliorate SI lesion-induced learning and memory impairments. Thereafter, doubts about selectivity of the neuronal systems involved and specificity of the actions of cholinergic drugs in animals have been raised (for

reviews, see 8–10, 15, 45). Likewise, the clinical benefit due to physostigmine in AD patients has been widely discussed (for reviews, see 8, 36, 45). Obviously, more direct and specific evidence was lacking.

However, the present results show that the cerebrovascular effects of physostigmine are not mediated by the SI neurons under resting conditions, unlike stimulation conditions. The highly reproducible lack of a differential effect of physostigmine with respect to the SI lesion indicates that physostigmine has a constant influence whatever the interindividual differences in pharmacological activation. The frontal predominance of the ChAT depletion corresponds to the pattern of the cerebrovascular responses to SI activation in conscious or α -chloralose-anesthetized rats (2, 31), but it seems less compatible with that of SI-lesioned rats, as mentioned above. The distribution of the respective effects of SI lesion and physostigmine corroborates the notion that the effects of cholinergic agents are less direct, unequivocal, and specific than expected.

Mechanisms Underlying the Results

Although physostigmine enhances cholinergic functioning (5), the influence of anticholinesterases is indirect and related to the level of presynaptic cholinergic activity, since they act by maintaining whatever Ach is present in the synaptic cleft. Therefore, the absence of differential effects of physostigmine may be due to (a) insufficient or inhibited Ach release, and/or to (b) physostigmine not acting on the cholinergic innominatocortical neurons.

(a) Several factors may lead to a lack of Ach at the cortical SI terminals. Insufficient (limited or subliminal) presynaptic activity of the cholinergic SI neurons (even on the unlesioned side) is consistent with the low tonic influence inferred from studies on lesioned animals and AD (see above). Physostigmine may in fact reduce the activity of SI neurons, by activating cholinergic inhibitory receptors on cell bodies. This idea has recently received support from experiments using nicotine microinjected into the SI (37). However, it is opposite to earlier data showing that Ach activates SI neurons (32).

At the level of the cortical cholinergic synapse, the fall in ChAT activity (presynaptic marker) and the loss of M2 presynaptic (inhibitory) autoreceptors, in both animal models and AD, together with the relative sparing of postsynaptic elements endowed with M1 muscarinic receptors (43), indicate that physostigmine should activate the innominatocortical synapse. The situation would be the same in the case of nicotinic receptors (32, 43). The recent findings of Flynn *et al.* (16) showing a reduced responsiveness of postsynaptic M1 muscarinic receptors in AD suggest an alternative explanation, but they require further support from animal studies.

Inhibition of the cholinergic transmission may also be due to noncholinergic systems. Several neurotransmit-

ters and neuromodulators interact with the cholinergic system and may considerably alter the effect of physostigmine (8). A balance with, e.g., the serotonergic system, might have been perturbed by the ibotenate lesion. GABAergic or peptidergic inputs to the cholinergic basal forebrain also appearing to exert an inhibitory, possibly tonic, influence have been shown to increase in AD (8, 45). They may more strongly inhibit the remaining cholinergic neurons. Thus, numerous interactions with the cholinergic transmission at various sites make it difficult to predict at present the effects of cholinesterase inhibition following short-term denervation.

(b) If physostigmine does not mainly act on the innominatocortical system, the observed cerebrovascular changes do not reflect activation of this system. (The several possibilities considered here are additional to those developed above, since they assume that there is no substantial Ach release from the SI projections.) In addition to functional studies showing cholinergic mediation of local CBF changes, there are biochemical (13, 18) and anatomical (1, 3, 11) data in favor of a cholinergic innervation reaching the cerebral microvasculature. However, few investigators have provided evidence for a direct cholinergic input from the basal forebrain to the cortical microcirculation (37). On the contrary, it is often suggested that an intrinsic cortical neuron is involved in the cortical cholinergic vasodilation (4, 18). Recent biochemical data also indicate that the cholinergic innervation of cortical microvessels does not directly originate in the basal forebrain (18), which is quite compatible with the present results. Above all, if this innervation were direct, physostigmine should plausibly have produced side-to-side differences on the cortical response.

Physostigmine may thus boost the activity of intrinsic cholinergic cortical neurons (4) that are thought to intervene in cholinergic vasodilation occurring through the basal forebrain (25), and it may also stimulate cholinergic systems indirectly projecting to the cortex (via a noncholinergic system), such as those originating from the mesopontine tegmental nuclei (35). The contribution of SI neurons to the physostigmine response would then appear negligible.

Two final possibilities are that physostigmine has a direct effect on the cerebral vasculature, or noncholinergic actions. There is, so far, no experimental evidence for a direct (myogenic) vascular mechanism of physostigmine (7), but noncholinergic cerebrovascular actions of physostigmine cannot be excluded (for references, see 36).

Significance and Implications for a Pharmacotherapeutic Approach

Despite the uncertainties about the mechanism of the cerebrovascular response to physostigmine and its site of action, this drug provides no evidence of enhance-

ment of the cortical cholinergic input originating in the SI. Since a metabolic activation would have undoubtedly resulted in a vasodilatation, it seems unlikely that physostigmine actually activates the cholinergic neurons of Meynert's nucleus, which casts doubts on its ability to specifically compensate a functional deficit of this nucleus in AD. The corollary of this statement is that physostigmine is not a presynaptic cholinergic functional marker, nor it is useful for detecting a defective Meynert's nucleus. This is at variance with the results showing that physostigmine restores the cerebral circulation of AD patients (19, 24), and that abnormalities in the cerebral circulation of AD patients are related to cholinergic dysfunction (22, 42). However, the time required for the onset of the degenerative process in AD is considerably longer than that presently allowed following SI lesion, and complex and interactive reorganization phenomena in the cholinergically dependent cortical circuitry, possibly including postsynaptic supersensitivity, can occur under more chronic conditions.

Although the cerebrovascular response to physostigmine was positive in both situations, the parallel between AD and the animal model suggests limited specificity in clinical improvements (24) as in behavioral changes (9, 10, 15). Consistently, using CBF investigation to quantify its pharmacodynamic effects, Geaney *et al.* (19) reported that physostigmine reversed the posterior parietotemporal hypoperfusion, and a similar focal localization of effects was observed by Gustafson *et al.* (21), whereas Hunter *et al.* (24) found the most significant effects in the frontal cortex, as did Honer *et al.* (22) using scopolamine. In addition to the mismatches in the cerebral distribution of biochemical or functional deficits cited above, these discrepancies could be explained by actions of physostigmine on some cortical postsynaptic target.

In conclusion, the cerebrovascular effects of physostigmine are not mediated through the basal forebrain but rather through the cortex, and the complex interplay of neurotransmitter and neuromodulator systems with the cholinergic system suggests that it could be likened to nootropic (postsynaptic), effects. Thus, the present findings do not support the relevance of physostigmine as a specific replacement-enhancement therapeutic agent to treat AD. However, despite the frequently advocated multiple-target pharmacological approach (as AD is a multisystem disorder), it is still necessary to explore therapies designed either to disinhibit the activity of the remaining cholinergic neurons of the basal forebrain, or to activate the presumably sensitized, postsynaptic cholinergic elements. Because of the insufficient knowledge of the mechanisms brought into play by cholinesterase inhibitors, there might be more suitable candidates than physostigmine for improving the functioning of a deficient Meynert's nucleus.

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