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Cerebral Blood Flow Responses to Somatosensory Stimulation Are Unaffected by Scopolamine in Unanesthetized Rat

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ABSTRACT

Studies with positron-emission tomography have indicated that muscarinic acetylcholine receptors may be involved in the mechanism of enhancement of cerebral blood flow (CBF) by neuronal functional activation. We examined the effects of muscarinic receptor blockade by scopolamine on the local CBF responses to vibrissal stimulation in the whisker-to-barrel cortex sensory pathway in unanesthetized rats. Local CBF was measured by the quantitative autoradiographic [^{14}C]iodoantipyrine method. Scopolamine (0.4 or 0.8 mg/kg) was injected i.v. 30 min before measurement of local CBF; control rats received equivalent volumes of physiological saline. Vibrissae on the left side of the face were stroked continuously throughout the 1-min period of measurement of CBF. Local CBF was determined bilaterally in four structures of the pathway, i.e., spinal and principal sensory trigeminal nuclei, ventral posteromedial thalamic nucleus, and barrel field of the sensory cortex, as well as in four representative structures unrelated to the pathway. The higher dose of scopolamine raised baseline CBF in the two trigeminal nuclei, but neither dose diminished the percentage of increases in local CBF because of vibrissal stimulation in any of the stations of the pathway. These results do not support involvement of muscarinic receptors in the mechanism of enhancement of local CBF by functional neuronal activation, at least not in the whisker-barrel cortex sensory pathway in the unanesthetized rat.

Roy and Sherrington (1890) first proposed an intrinsic regulation of the cerebral circulation that adjusts local cerebral blood flow (CBF) to the altered metabolic demands associated with local alterations in functional activity in the tissue. Since then, there have been many demonstrations that neural functional activation does indeed increase local energy metabolism and CBF in the activated areas. Despite intensive efforts that revealed many factors capable of influencing cerebral blood vessels and CBF (Kuschinsky and Wahl, 1978; Faraci and Heistad, 1998), the mechanisms responsible for the function-related changes in local CBF have never been unequivocally defined. Various possible mechanisms have been considered, e.g., direct neurogenic control of vascular tone, chemical factors related to metabolism, neurotransmitters and neuromodulators released from neurons by functional activity, nitric oxide, adenosine, and prostaglandins (Kuschinsky and Wahl, 1978; Edvinsson et al., 1993; Faraci and Heistad, 1998). Cholinergic fibers of parasympathetic origin and nerve terminals of intrinsic cholinergic neural pathways surround some of the cerebral vessels (Duckles, 1981; Estrada and Krause, 1982; Estrada et al., 1983; Saito et al., 1985; Suzuki et al., 1990; Edvinsson et al., 1993), and acetylcholine (ACh) is known to dilate blood vessels by an endothelial muscarinic receptor-dependent stimulation of the synthesis of the endothelium-derived relaxing factor (Furchgott and Zawadzki, 1980) now known to be nitric oxide (Ignarro et al., 1987). Cholinergic mechanisms are therefore reasonable candidates as mediators of the functional activation of local CBF.

Ogawa et al. (1994) recently reported results of studies with positron-emission tomography (PET) that support a role for cholinergic muscarinic mechanisms in the enhancement of CBF by neural functional activation. They found that the increases in local CBF in the somatosensory cortex normally observed in anesthetized cats during vibrotactile stimulation of the forepaw were totally abolished by i.v. administration of the muscarinic antagonist scopolamine without any effect on local cerebral glucose utilization. Subsequently, Tsukada et al. (1997a,b, 1998), also using PET, reported that scopolamine also blocked the local CBF response in the somatosensory cortex to vibrotactile stimulation of the hand in conscious rhesus monkeys, and that the anticholinesterase drug physostigmine reversed this effect of scopolamine.

We have been using the quantitative autoradiographic [^{14}C]iodoantipyrine ([^{14}C]IAP) method, which provides much better spatial resolution than PET methods, to study the

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ABBREVIATIONS: CBF, cerebral blood flow; ACh, acetylcholine; [^{14}C]IAP, [^{14}C]iodoantipyrine, 4-iodo[*N-methyl*-^{14}C]antipyrine; PET, positron-emission tomography; NOS, nitric oxide synthase.

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effects of vibrissal stimulation on local CBF in the whisker-barrel sensory pathway (Woolsey and Van der Loos, 1970; Ginsberg et al., 1987; Adachi et al., 1994). Whisker stroking has been shown to result in substantial increases in local CBF in four stations of the pathway examined (Ginsberg et al., 1987; Adachi et al., 1994). In similar previous studies, we (Adachi et al., 1994) found that almost total inhibition of nitric oxide synthase (NOS) activity in the brain did not alter the percentage of increases in local CBF induced by vibrissal stimulation in any of the stations of this pathway in conscious rats. These results did not, however, exclude the possibility of a role for muscarinic receptors not coupled to nitric oxide synthesis. In view of the results obtained with PET methods, we have examined the effects of scopolamine on the increases in local CBF induced by functional activation of the whisker-barrel sensory cortex in unanesthetized rats. In doses that produced clear evidence of behavioral effects and were equal to or greater than those known to alter local cerebral energy metabolism, scopolamine administration did not diminish the magnitude of the increases in local CBF caused by functional activation.

Materials and Methods

Chemicals. Chemicals were purchased from the following sources: (−)-Scopolamine hydrobromide (Sigma Chemical Co., St. Louis, MO), and 4-iodo[*N-methyl*-^{14}C]antipyrine ([^{14}C]IAP; specific activity, 54 mCi/mmol; DuPont-NEN, Boston, MA).

Animals. Normal adult male Sprague-Dawley rats (300–425 g) were purchased from Charles River Laboratories (Wilmington, MA) and maintained in a climate-controlled room on a normal 12-h light/dark cycle, with food and water available ad libitum. They were fasted but allowed free access to water for 16 h immediately before the experiment. The rats were anesthetized with halothane (5% for induction and 1.0–1.5% for maintenance) in 70% N_2O/30% O_2. Polyethylene catheters (PE 50, Clay-Adams, Parsippany, NJ) were inserted into both femoral arteries and the left femoral vein. One arterial catheter was for continuous recording of mean arterial blood pressure; the other was used for sampling of arterial blood and fixed at precisely 16 cm in length to allow standardized correction for catheter dead-space washout. The venous catheter was used for injection of drugs and tracer. After closure, the surgical wounds were treated with 5% lidocaine ointment. Duration of anesthesia was 25 to 30 min. After completion of the surgical procedure, a loose-fitting plaster cast was applied to the pelvic area and taped to a lead brick to prevent locomotion of the rats. Body temperature was continuously monitored by a rectal probe and maintained at 37°C by thermostatically controlled heating lamps (model 73A; Yellow Springs Instrument Co., Inc., Yellow Springs, OH). At least 3 h were then allowed for the rats to recover from the anesthesia before initiation of the procedure for measurement of local CBF. All the procedures performed on the animals were in strict accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and approved by the local Animal Care and Use Committee.

Monitoring of Physiological Variables. Three hours after surgery and periodically thereafter, several physiological variables of relevance to the cerebral circulation were measured to assess the status of the animal before and during the experimentally induced conditions. Mean arterial blood pressure was monitored with a blood pressure analyzer (Micro-Med, Inc., Louisville, KY) that had been calibrated with an air-damped mercury manometer. Arterial blood $pACO_2$, pAO_2, pH, and bicarbonate concentration were measured with a blood-gas analyzer (model 288 Blood Gas System; Ciba-Corning Diagnostics Corp., Medfield, MA).

Measurement of Local CBF. Local CBF was determined by the quantitative autoradiographic [^{14}C]IAP method of Sakurada et al. (1978) as modified by Adachi et al. (1994). The duration of the measuring period was approximately 1 min, and corrections for delay and washout of the arterial catheter sampling system were incorporated in the computation of blood flow as described previously (Freygang and Sokoloff, 1958). To minimize the magnitude of these corrections, blood flow through the arterial catheter was adjusted to greater than 40 dead-space vol/min.

Experimental Procedures. Three hours after recovery from anesthesia, the vibrissae on the right side of the face of all animals were clipped flush with the skin. This was done because unanesthetized rats tend to move their heads and spuriously stimulate whiskers on both sides of the face. It was therefore necessary to clip the whiskers on the control side to achieve truly unstimulated control conditions. Such clipping does result in slight (5–10%) but statistically significant reductions in blood flow in structures of the pathway on the control side, but these reductions are detectable only in quantitative measurements of blood flow and are not readily visible in the autoradiographic images (see Fig. 1 in Adachi et al., 1994). Scopolamine-treated animals were then infused i.v. with scopolamine dissolved in normal saline in concentrations of 0.4 or 0.8 mg/ml over 1 min; the total dose was either 0.4 or 0.8 mg/kg. Control animals were infused with equivalent volumes of normal saline. Thirty minutes after the scopolamine or saline administration and beginning simultaneously with the onset of infusion of the [^{14}C]IAP, the whiskers on the unclipped left side of the face were continuously stroked with a soft paintbrush at a frequency of 2 to 3 strokes/s. This unilateral vibrissal stimulation was continued throughout the 1-min period of measurement of local CBF.

Four bilateral structures in the whisker-barrel cortex pathway were selected for determination of local CBF. These were the spinal trigeminal nucleus, the principal sensory trigeminal nucleus, the ventral posteromedial nucleus of the thalamus, and the barrel field of the somatosensory cortex, which were identified in the autoradiograms by comparison with thionine-stained brain sections corresponding to the autoradiograms and the rat brain atlas of Paxinos and Watson (1997). Local CBF in the structures of the pathway on the stimulated side were compared with the values in the corresponding structures on the opposite side, which served as unstimulated controls. In addition, four other structures unrelated to this sensory pathway were examined bilaterally for possible nonspecific effects of scopolamine and/or vibrissal stimulation; these included cerebellar white matter, caudate-putamen, primary motor cortex, and nucleus accumbens (Paxinos and Watson, 1997), and local CBF was determined separately for the stimulated and unstimulated sides.

Statistical Analyses. Data are presented as means ± S.E. Statistical significance of differences in local CBF between stimulated and unstimulated sides were determined by paired t tests. The significance of the effects of scopolamine on values of local CBF in both the stimulated and unstimulated structures compared with values in corresponding structures in the saline control animals was evaluated by Dunnett's test. To test the homogeneity of the three groups, i.e., saline treated and those given 0.4 and 0.8 mg/kg of scopolamine, with respect to percent difference in CBF between unstimulated and stimulated sides for each structure, the Kruskal-Wallis test (one-way ANOVA by ranks) was used. If statistical significance ($p < .05$) was found in the percentage of differences, the nonparametric multiple-comparison procedure of Steel (1959) for comparing several treatment groups with a control group, as modified and described by Hochberg and Tamhane (1987), was used.

Results

Effects of Scopolamine on Behavior and Physiological Variables. All of the scopolamine-treated rats exhibited restlessness and agitation, as well as rigidity and curling of the tail, which were enhanced by touch. There were no obvi-

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ous differences in behavior in the rats given 0.4 and 0.8 mg/kg of scopolamine. The behavioral effects appeared immediately after scopolamine administration and persisted throughout the procedure for determination of local CBF. Arterial blood pressure, $pACO_2$, pAO_2, pH, and bicarbonate concentration at the time of the CBF determinations were essentially the same in the saline- and scopolamine-treated rats (Table 1) and remained stable and within normal ranges throughout the experiment.

Effects of Scopolamine on Baseline CBF. The values of CBF in the four structures of the whisker-barrel cortex pathway in the unstimulated control side of the brain, as well as in the four structures unrelated to the pathway, were used to assess the effects of scopolamine per se on baseline CBF. Only the higher dose (0.8 mg/kg) of scopolamine increased the baseline CBF in unstimulated structures of the pathway compared with the corresponding values in the saline-treated controls; these increases were observed only in the two trigeminal nuclei in the spinal cord and brain stem (Fig. 1). No significant effects of scopolamine on baseline CBF were observed in any of the other structures within or outside the whisker-barrel pathway examined (Fig. 1 and Table 2).

Effects of Scopolamine on Responses of Local CBF to Functional Activation. Unilateral vibrissal stimulation resulted in marked increases in CBF in all four stations of the functionally activated whisker-barrel pathway, i.e., ipsilateral spinal and principal sensory trigeminal nuclei and contralateral thalamic ventral posteromedial nucleus and barrel field of the somatosensory cortex, above the values in the homologous structures of the unstimulated side (Fig. 1). These effects of vibrissal stimulation on CBF were clearly visible in the autoradiographic functional brain images (Fig. 2). The vibrissal stimulation had no effects on CBF in any of the four representative structures unrelated to the whisker-barrel pathway that were examined (Table 2).

The higher dose of scopolamine (0.8 mg/kg) raised CBF statistically significantly in both the unstimulated and stimulated principal sensory trigeminal nuclei above the levels in the corresponding nuclei of the saline-treated controls, but neither dose of scopolamine had any significant effect on the percentage of increases in CBF evoked by functional activation in any of the four stations of the stimulated pathway (Fig. 1 and Table 3). The lack of effect of scopolamine on the differences in CBF between the stimulated and unstimulated sides is apparent in the functional brain images (Fig. 2).

TABLE 1

Physiological variables immediately before CBF measurement

Values are means ± S.E. of *n* animals. No statistically significant differences between scopolamine-treated and control animals for any of the variables.

Physiological Variable	Saline Controls	Scopolamine	
		0.4 mg/kg	0.8 mg/kg
n	7	6	5
Mean arterial blood pressure (mm Hg)	107 ± 2	110 ± 2	110 ± 2
$pACO_2$ (mm Hg)	36 ± 1	35 ± 1	36 ± 1
pAO_2 (mm Hg)	90 ± 3	90 ± 2	91 ± 3
Arterial pH	7.44 ± 0.01	7.44 ± 0.01	7.43 ± 0.01
Arterial HCO_3^- concentration (mM)	26.0 ± 0.4	25.0 ± 0.4	25.0 ± 0.4

Discussion

There have been many demonstrations that functional activation of neural pathways in the brain results in increases in CBF in structural and functional components of those pathways. Although its mechanism is still undefined, this cerebral vascular response to functional activation is currently widely used to map functional neural pathways involved in cognitive functions. Our results confirm once again that, in the conscious rat, functional activation of the whisker-barrel cortex sensory pathway evokes marked increases in CBF in all stations of the pathway examined. Scopolamine, however, in doses sufficient to evoke obvious behavioral effects, had no effects on the increases in local CBF elicited by the vibrissal stimulation. These results therefore provide no evidence to support a role for muscarinic receptors in the mechanism of functional activation of local CBF and are in contrast to the reported suppression of CBF responses to vibrotactile stimulation in the sensory cortex of cats and monkeys by scopolamine (Ogawa et al., 1994; Tsukada et al., 1997a,b, 1998). They are, however, consistent with the results of studies in which i.v. and topically applied atropine as well as histologically confirmed cholinergic denervation of the sensory cortex by lesions of the nucleus basalis magnocellularis had no significant effects on pial arteriolar dilation evoked by sciatic nerve stimulation (Ibayashi et al., 1991).

We have no definitive explanation for this discrepancy, but there are several possibilities to be considered. First, there is the question of species differences; our studies were done in rats and the others were done in cats and monkeys. This is an unlikely explanation, however, because rats also have cholinergic innervations from the nucleus basalis magnocellularis to blood vessels and neurons in many regions of the brain, including the cerebral cortex (Vaucher and Hamel, 1995; Vaucher et al., 1997). Another possibility is that the doses of scopolamine we used were insufficient to provide effective concentrations in the brain. Ogawa et al. (1994) used 0.35 mg/kg in the cat, and Tsukada et al. (1997a,b, 1998) used 0.001 to 0.5 mg/kg in the monkey, whereas we used 0.4 and 0.8 mg/kg in the rat. Even our lower dose, however, has been shown by Weinberger et al. (1979) to produce widespread, marked decreases in cerebral glucose utilization throughout the brain, including the thalamus and cerebral cortex. Furthermore, the dose of 0.4 mg/kg produced prominent behavioral changes indicative of central nervous system effects, and even when we doubled that dose, we still found no attenuation of the increases in CBF evoked by vibrissal stimulation. It is therefore unlikely that the dosage of scopolamine explains the discrepancy.

It is more likely that the discrepancy is due to methodological differences. Our experiments were carried out in rats that had been surgically prepared under relatively light halothane anesthesia but studied at least 3 h after recovery from the anesthesia. We chose this anesthetic agent and recovery time because, in many studies over many years, we found that rats exhibit no residual effects on local CBF or metabolism by 3 h after recovery from the halothane anesthesia. On the other hand, we observed that ketamine, the agent used by Tsukada et al. (1971, 1997a,b, 1998) in the preparation of their monkeys, produces pronounced effects on local cerebral glucose utilization, particularly in limbic and sensory pathways (Crosby et al., 1982), that persist for at least 24 h even

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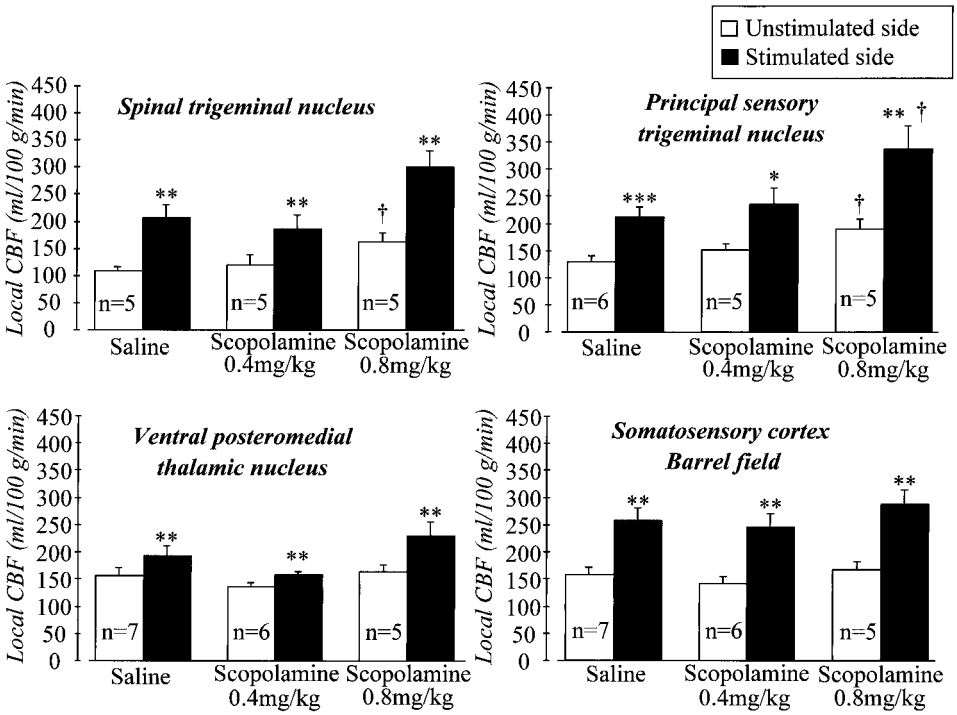

Fig. 1. Effects of i.v. administration of 0.4 or 0.8 mg/kg of scopolamine on blood flow in four stations of the whisker-barrel cortex somatosensory pathway in both the unstimulated and stimulated sides of the brain during unilateral vibrissal stimulation. The whiskers on the left side of the face were continuously stroked during the period of blood flow measurement. Column heights and error bars represent mean rates of local CBF ± S.E. obtained from the number of rats indicated. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$, by paired comparison with unstimulated side. $^{\dagger}p < .05$, compared with corresponding side of saline-treated controls by Dunnett's test for multiple comparisons with common control.

TABLE 2

Effects of scopolamine on CBF in representative cerebral structures unrelated to whisker-barrel pathway

Values are means ± S.E. of n animals.

Structures and Treatments	n	Left	Right	Side to Side
		$ml \cdot 100\ g^{-1} \cdot min^{-1}$		*%Difference**
Cerebellar white matter				
Saline control	7	42 ± 7	39 ± 4	−4 ± 4
Scopolamine				
0.4 mg/kg	6	40 ± 3	42 ± 4	6 ± 5
0.8 mg/kg	5	48 ± 4	49 ± 3	2 ± 4
Caudate putamen				
Saline control	6	139 ± 13	137 ± 11	−1 ± 2
Scopolamine				
0.4 mg/kg	6	154 ± 12	155 ± 13	1 ± 3
0.8 mg/kg	5	178 ± 17	175 ± 17	−2 ± 2
Primary motor cortex				
Saline control	6	152 ± 12	153 ± 7	2 ± 4
Scopolamine				
0.4 mg/kg	5	161 ± 16	186 ± 18	18 ± 8
0.8 mg/kg	5	166 ± 17	185 ± 25	11 ± 4
Nucleus accumbens core				
Saline control	6	136 ± 9	139 ± 9	3 ± 1
Scopolamine				
0.4 mg/kg	6	134 ± 5	136 ± 5	2 ± 3
0.8 mg/kg	5	153 ± 10	159 ± 9	5 ± 4

* Mean of percentage of differences between right and left sides obtained in each of the rats and not difference between means. No statistically significant effects of either dose of scopolamine on local blood flow and no statistically significant bilateral differences in CBF in any of the structures.

though the animals are then conscious and exhibit no obvious gross behavioral effects (our unpublished data). Furthermore, it was recently reported that, at least in rats, ketamine diminishes spontaneous firing in sensorimotor striatal neurons and abolishes their discharges in response to cutaneous stimulation for at least 5 h after its administration, a time when the animals behaviorally appear to have recovered from the anesthetic (West, 1998). It is conceivable that such persistent effects of ketamine might have interacted with those of scopolamine and contributed to the observed inhibition in CBF response to vibrotactile stimulation. Ogawa et al. (1994) studied the effects of forepaw stimulation in cats under pentobarbital anesthesia. Ueki et al. (1992), however, have reported that pentobarbital reduces the evoked electrical potential and eliminates the metabolic activation in the sensory cortex in response to forepaw stimulation, and Lindauer et al. (1993), studying the effects of vibrissal stimulation on CBF changes in the sensory cortex of the rat, concluded that barbiturate anesthesia "does not seem suited for CBF coupling studies due to low amplitude of response".

There are also other important methodological differences. Although the autoradiographic [^{14}C]IAP and the PET-based $H_2{}^{15}O$ methods are based on the same physical principles, they implement these principles quite differently. Both techniques assume homogeneous tissue compartments with respect to blood flow and tracer solubility, an assumption that is difficult to satisfy in tissues as heterogeneous as brain. The validity of this assumption can, however, be approached by the autoradiographic technique, which has a spatial resolution of about 200 μm and in which the regions of interest to be examined are directly visualized and circumscribed on the basis of relatively uniform optical density in the autoradiograms. The 200-μm resolution was determined by measurement of the line-spread function of the isotope; it represents twice the full width at half-maximum of this line function (Smith, 1983). In contrast, the full width at half-maximum of the PET scanners used in the studies of Ogawa et al. (1994) and Tsukada et al. (1971, 1997b, 1998) was at best about 3 mm greater than that of the autoradiographic technique by more than an order of magnitude. Accurate quantification is then possible only in homogeneous structures larger than twice the full width at half-maximum (Hoffman et al., 1979), e.g., greater than 6 mm, conditions not easily met in cat and monkey brains. Otherwise, the results reflect the decreased sensitivity resulting from partial volume effects and the con-

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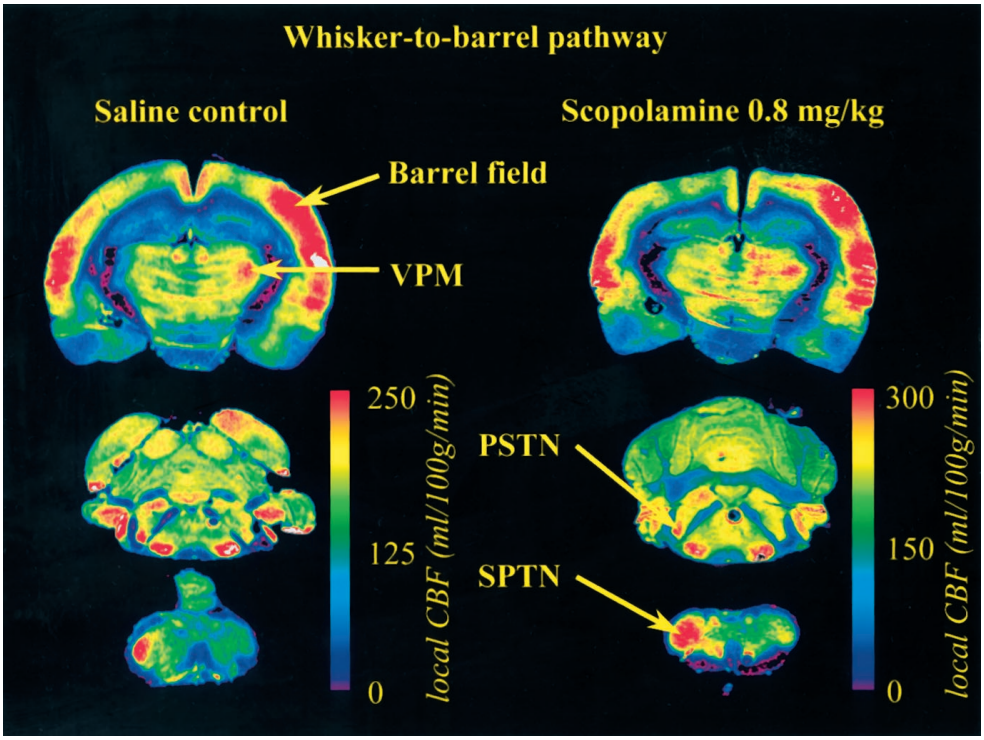

Fig. 2. Computerized image-processed [^{14}C]IAP autoradiograms in which the level of local CBF is quantitatively encoded into a color scale. Note the marked effects of unilateral vibrissal stimulation on local blood flow in the four stations of the whisker-barrel cortex pathway in both saline control rats and rats pretreated with 0.8 mg of scopolamine.

TABLE 3

Effects of scopolamine on percentage of increases in local CBF (percentage of differences between stimulated and unstimulated sides) in whisker-barrel cortex pathway during vibrissal stimulation

Values are means of individual percentage of differences ± S.E. of *n* animals, and not percentage of differences between means.

Structures	Saline Control	Scopolamine	
		0.4 mg/kg	0.8 mg/kg
	% Difference		
Spinal trigeminal nucleus	88 ± 14 (n = 5)	59 ± 12 (n = 5)	86 ± 11 (n = 5)
Principal sensory trigeminal nucleus	64 ± 9 (n = 6)	56 ± 13 (n = 5)	76 ± 6 (n = 5)
Ventral posteromedial thalamic nucleus	22 ± 5 (n = 7)	17 ± 4 (n = 6)	39 ± 6 (n = 5)
Barrel field of somatosensory cortex	64 ± 13 (n = 7)	79 ± 18 (n = 6)	73 ± 8 (n = 5)

Neither dose of scopolamine had any statistically significant effect on percentage of increases in local CBF due to stimulation.

sequences of applying a method that assumes homogeneous compartments to a heterogeneous mixture of tissues.

Our results do not completely exclude a role for cholinergic mechanisms in the regulation of the cerebral circulation. They only provide evidence against a role for muscarinic receptors in the functional activation of CBF. Intracerebral arterioles and microvessels isolated from cerebral and cerebellar cortex and caudate nucleus have been shown to contain choline acetyltransferase activity and binding sites for specific muscarinic ligands (Estrada and Krause, 1982; Estrada et al., 1983; Moro et al., 1995). In the rat, however, cerebral cortex cholinergic fibers do not make direct contact with the outer basal lamina of the vessels, although it is possible that their terminals may be close enough to intraparenchymal blood vessels to act on them in a paracrine manner (Chédotal et al., 1994). Cholinergic fibers of parasympathetic origin have been demonstrated on large pial arteries but not yet on resistance vessels in the parenchyma (Suzuki et al., 1990). The functional significance of these cholinergic innervations and muscarinic binding sites on the cerebral vessels in the regulation of CBF is, however, uncertain. They may well be involved in adjustments of steady-state or baseline levels of overall CBF without directly mediating the changes in local CBF in response to alterations in local neuronal functional activity. For example, ACh stimulates endothelial nitric oxide synthesis by a muscarinic receptor mechanism (Furchgott and Zawadzki, 1980; Ignarro et al., 1987; Rubanyi, 1991; Dauphin et al., 1994), and inhibition of NOS activity leads to cerebral vasoconstriction and decreased CBF throughout the brain but without altering the local CBF response to functional activation in unanesthetized rats (Wang et al., 1992; Adachi et al., 1994). Nitric oxide can be synthesized in brain by at least three isoforms of NOS: one in the endothelium, an inducible one in the glia, and the third exclusive to neurons. Neuronal NOS was the one that was implicated in the functional activation of local CBF (Ayata et al., 1996), but it was then conceded by the same group that the nitric oxide formed by it does not fully account for the functional activation of local CBF and that it may be "acting as a permissive factor rather than a mediator" (Ma et al., 1996).

There may be mechanisms other than direct cholinergic innervation to the cerebral blood vessels. There are intrinsic cholinergic synapses within the brain, and ACh released from these synapses might act locally on microvessels in the immediate vicinity. Alternatively, there may be cholinergic synapses that are in multisynaptic pathways that ultimately project to the blood vessels but release other transmitters. These cholinergic synapses may use nicotinic receptors, which are prevalent in brain. The results of our experiments with scopolamine exclude a role for muscarinic receptors in the functional activation of CBF, at least in the whisker-barrel cortex pathway of the unanesthetized rat, but they do

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not eliminate the possibility of other cholinergic mechanisms involving nicotinic receptors and/or other sensory pathways.

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