Response of Local Blood Flow in the Caudate Nucleus of the Cat to Intraventricular Administration of Carbachol

Gaspard De Ley, V.M.D., Jaak Weyne, M.D., Gerard Demeester, M.S., and Isidoor Leusen, M.D.

SUMMARY The effect of perfusion of the cerebral ventricles with artificial cerebrospinal fluid containing carbachol on the blood flow in the caudate nucleus of the cat and the possibility to inhibit this effect by anticholinergic drugs was studied by means of the hydrogen clearance technique. After a control period during which both lateral ventricles were perfused with artificial CSF of identical composition, the drug under study was added on one side (experimental side) while the other side continued to be perfused with the control artificial CSF (control side). The blood flow on the experimental side and on the control side were compared. A dose dependent response to carbachol was observed. Lower concentrations of carbachol (10^-4 up to 10^-3M) caused vasodilatation whereas high concentrations (10^-2M) caused local vasoconstriction. The increase in the local blood flow caused by the low carbachol concentrations was reduced by both atropine (10^-5M) and hexamethonium (10^-3M). The fall in CBF observed with the high carbachol concentration was prevented by atropine (10^-5M). It may be concluded that low, physiologically more meaningful, carbachol concentrations cause a local vasodilation due to interaction with both muscarinic and nicotinic receptors.

The presence of muscarinic receptors has drawn attention to the possible influence of cholinergic innervation on the regulation of cerebral blood flow. Evidence for the existence of such a cholinergic innervation in the pial vessels was deduced from histochemical or biochemical measurements of cholinesterase, cholineacetyltransferase and choline uptake. It was also reported that cholinergic innervation in the intraparenchymatous cerebral vessels. It was also reported that cholinergic innervation of the intraparenchymal cerebral vessels, which is well established for pial arteries, apparently does not extend to the intraparenchymal arteries, although more recent evidence would suggest that they do. Aubineau et al observed in rabbits that infusion with carbachol increased blood flow in the caudate nucleus and that this effect was antagonized by atropine. The decrease of local cerebral blood flow following postganglionic stimulation of the cervical sympathetic chain above the superior cervical ganglion was reduced during the infusion with carbachol; this effect was not affected by atropine.

The aim of the present study was to examine the effect of perivascular application of the cholinergic agonist carbachol on the local cerebral blood flow in the caudate nucleus of the cat.

Methods
Cats of either sex weighing approximately 3 kg were anesthetized with ether followed by an intravenous dose of sodium-thiopental (Pentobarbital) (30 mg/kg). A tracheostomy was performed and the right femoral artery and vein were cannulated for blood pressure measurement and the administration of drugs respectively. The head was fixed in a stereotaxic apparatus, the scull was exposed and appropriate holes were made by means of a dental drill. Relaxation and anesthesia were...
maintained by gallamine (5 mg/kg every hour) and artificial ventilation with nitrous oxide (30% O₂, 70% N₂O). Blood gases were controlled and the animals were kept in steady state normocapnia (Pa₇CO₂ 30-40 mm Hg) by adjusting the ventilation. Blood pressure was monitored throughout the experiment; in the experiments reported mean blood pressure was at least 100 mm Hg.

Bilateral ventriculocisternal perfusion (VCP) was installed as described previously.²⁶,²⁷ Two inlet cannulas, one on each side, were lowered into the lateral cerebral ventricles by means of a microdrive system. Through each cannula, artificial cerebrospinal fluid (CSF) was administered at the rate of 0.123 ml/min.

An outlet cannula was introduced into the suboccipital cistern. The composition of the artificial CSF was as follows (mmol/l): NaCl 138; KCl 3.3; NaHCO₃ 25.0; NaH₂PO₄·H₂O 0.5; MgCl₂·6H₂O 1.2; CaCl₂ 1.25; glucose·H₂O 3.1.

The substances under study were added to the artificial CSF. The osmolality and the bicarbonate concentration were carefully checked and when necessary adjusted to 320 mOsm/kg and 25 mmol/l respectively.

**Blood Flow Measurement**

Blood flow in both caudate nuclei was measured simultaneously by means of the hydrogen clearance method.²⁸-²⁹ A hydrogen sensitive electrode (glass insulated platinum iridium wire ø 0.35 mm) was placed stereotaxically in the head of each caudate nucleus (coordinates A_13; L_4, H_0 according to the stereotaxic atlas of Snider and Niemer). The animals were saturated with hydrogen by adding 10% hydrogen gas to the inspired air. The administration of hydrogen was discontinued after a saturation period of at least 10 minutes.

The local CBF was calculated from the hydrogen desaturation curves in a semi-automatized manner as described earlier.³⁰

The blood flow in both caudate nuclei was measured simultaneously and the “blood flow ratio E/C” (flow on the experimental side/flow on the control side, cf. below) was calculated.

**Experimental Design**

Each experiment started with a “control period” during which both lateral ventricles were perfused with mock CSF of the same composition (pure or with drugs added). Three control flow measurements were made at intervals of 45 min. The first flow measurement was made one hour after the start of the ventriculocisternal perfusion.

After the third control measurement, the composition of the perfusion fluid was changed on one side (experimental side: E) whereas on the other side (control side: C) perfusion was continued with the same solution as during the control period (t = 0). During the “experimental period” three or four flow measurements were made at intervals of 45 min.

**Effects of Carbachol**

Several series of experiments were performed.

After a control period of symmetrical perfusion with “pure” mock CSF, carbachol was added to the perfusion fluid on the experimental side in concentrations of 10⁻³ (n = 7) and 10⁻³ M (n = 3). Low (10⁻⁴M) and high (10⁻³M) carbachol concentrations were tested in separate groups of animals. During preliminary experiments carbachol concentrations of 10⁻⁴M as well as 10⁻³M were tested in a few animals.

In another series of experiments the effects of carbachol were studied following the administration of atropine. After a control period of symmetrical perfusion with mock CSF containing atropine (10⁻⁵M), carbachol 10⁻³M (n = 8) or 10⁻⁴M (n = 5) was added to the perfusion fluid on the experimental side. Low (10⁻³M) and high (10⁻²M) carbachol concentrations were tested in separate groups of animals.

The effect of carbachol (10⁻³M) was also examined in the presence of hexamethonium. After a control period of symmetrical perfusion with mock CSF containing hexamethonium 10⁻³M (n = 7), carbachol 10⁻³M was added to the perfusion fluid on the experimental side. The relative concentrations of the antagonists (atropine or hexamethonium) versus the agonist (carbachol) were deduced from in vitro studies.

In order to evaluate the results the blood flow ratio (R = E/C) at the end of the control period (t = 0) and at the end of the experimental period (t = 135 min and t = 180 min for the high and low concentrations of carbachol respectively) were compared. The results were analyzed statistically by means of the t-test for paired observations.

**Results**

**Effect of Different Concentrations of Carbachol on Blood Flow in the Caudate Nucleus**

The effect of a concentration of 10⁻⁴M carbachol was studied in a group of seven animals. The mean values of blood flow in the control period varied between 81 and 74 ml·min⁻¹·100 g⁻¹ on the control side and between 74 and 71 ml·min⁻¹·100 g⁻¹ on the experimental side (table 1). The addition of carbachol (10⁻³M) caused an increase of the blood flow in the caudate nucleus on the experimental side of about 25%, whereas the blood flow in the caudate nucleus on the control side remained practically unchanged. The blood flow ratio (R = E/C) increased from 0.96 ± 0.14 at t = 0 to 1.27 ± 0.13 at t = 180 (p < 0.01), which indicates that there was vasodilatation on the experimental side (fig. 1A).

The addition of 10⁻⁴ or 10⁻³M carbachol similarly produced vasodilatation, but the effect was much more pronounced.

The addition of 10⁻³M carbachol to the ventricular perfusate did not produce any systemic cardiovascular effects so that the general hemodynamic parameters did not change throughout the experiment.

The effect of a high carbachol concentration was tested in another group of animals. After adding 10⁻³M carbachol, the blood flow in the caudate nucleus on the
The ratio was 0.88 ± 0.08 at t = 0 and 0.97 ± 0.09 experimental side in contact with carbachol (fig. 1B). Control sides of the individual animals showed a small increase indicating some resting vasodilatation on the control side only a slight decrease was observed. The resulting blood flow ratio (R = E/C) was 0.99 ± 0.16 at t = 0 and 0.95 ± 0.21 (no significant difference) (fig. 2A). This large dose of carbachol was generally badly tolerated by the animals and caused a gradual but important fall in blood pressure. The results in table 1 bear upon three methods of carbachol (10−3 M) to the experimental side, the blood flow ratio changed from 0.81 ± 0.08 at t = 0 to 0.89 ± 0.09 at t = 180 (no significant difference) (fig. 1C). The effect of the high carbachol concentration (10−3 M) was also inhibited by atropine. When both lateral ventricles were perfused with artificial CSF containing atropine (10−5 M), the blood flow in the caudate nuclei varied between 116 and 107 ml-min−1·100 g−1 on the control side and between 91 and 85 ml-min−1·100 g−1 on the experimental side. After adding carbachol (10−3 M) to the experimental side, the blood flow ratio changed from 0.81 ± 0.08 at t = 0 to 0.89 ± 0.09 at t = 180 (no significant difference) (fig. 1C).

Discussion

The experiments show that the addition of carbachol to the ventricular fluid affects blood flow in the adjacent caudate nucleus. Since CBF measurements were made before and after adding carbachol, each animal served as its own control; moreover the asymmetrical perfusion and the simultaneous CBF measurement on both sides allowed to differentiate between local effects of carbachol and more general effects.

The experiments show that ventriculo-cisternal perfusion with low concentrations of carbachol causes blood flow in the caudate nucleus to increase as a result of local vasodilatation.

The optimal vasodilatory effect was obtained with carbachol 10−3M whereas a lower (10−5 M) or a higher (10−4 M) concentration still produced vasodilatation to a lesser degree. From a quantitative point of view there seems to be a discrepancy between our results and the results obtained by other investigators on in vitro preparations or on perivascular application on pial vessels. They observed vasodilatation at much lower ace-

### Table 1

<table>
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<th>Time (min)</th>
<th>−90</th>
<th>−45</th>
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<tr>
<td>A (n = 7)</td>
<td>C</td>
<td>79 ± 12</td>
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<td>81 ± 13</td>
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<td>71 ± 10</td>
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<td>97 ± 12</td>
<td>93 ± 13</td>
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<td>108 ± 17</td>
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<td>114 ± 19</td>
<td>121 ± 19</td>
</tr>
<tr>
<td>C (n = 7)</td>
<td>C</td>
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<td>110 ± 16</td>
<td>107 ± 14</td>
<td>104 ± 12</td>
<td>107 ± 11</td>
<td>101 ± 10</td>
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<tr>
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<td>91 ± 7</td>
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<td>99 ± 12</td>
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Mean ± SEM. At t = 0, carbachol 10−5 M (upper part) or carbachol 10−3 M (lower part) was added to the perfusion fluid of the experimental side. Composition of the "control fluid" (CF) in A: mock CSF; in B: mock CSF containing atropine (10−5 M) and in C: mock CSF containing hexamethonium (10−3 M).
only approximately 15% of the concentration of histamine in the irrigation fluid.32

Experiments in which very large doses of carbachol (10^-3M) were used, showed a manifest decrease of the blood flow in the caudate nucleus due to local vasoconstriction. Our in vivo experiments confirm the dual effect observed on in vitro preparations: vasodilatation of isolated segments of cerebral arteries with low concentrations (up to 10^-4M) of acetylcholine and vasoconstriction with high concentrations (> 10^-4M).1 The high concentrations, however, caused general circulatory changes (blood pressure fall) in most animals; in the three experiments described the mean arterial blood pressure remained higher than 100 mm Hg throughout the experiment.

The effect of the classical anticholinergic drugs allows to speculate about the receptor type involved. Atropine (10^-5M) clearly impedes the dilatation caused by carbachol (10^-3M) as well as the vasoconstriction caused by carbachol (10^-3M). When administered intraventricularly after treatment with atropine, carbachol (10^-3M) only produced a small and insignificant vasodilatation.

Our experiments thus show that muscarinic recep-

5ylcholine (or carbachol) concentrations than the ones we used. Several factors can explain the need for higher concentrations in our experiments. Firstly, the concentration of carbachol in the ventricular CSF was undoubtedly lower than in the infused artificial CSF because of the addition of freshly secreted endogenous CSF. Secondly, carbachol had to diffuse into the periventricular brain tissue before its effect on the blood flow in the caudate nucleus could be detected by the method used in the experiments. Because of this diffusion gradient the concentration of carbachol in the extracellular fluid of the caudate nucleus where the blood flow was measured, can be accepted to be much lower than that in the ventricular CSF. For histamine it was found that after 1 hour of epiarchnoid irrigation, the mean concentration of exogenous histamine in the first mm of the brain subjacent to the irrigating solution was
tors are involved in the dilator as well as in the constrictor response to carbachol. In this respect our results confirm the experimental data concerning the effect of cholinomimetics on isolated cerebral blood vessels and on pial vessels reported in the literature and they extend those observations to the deeper parenchymal blood vessels. Our findings are also in line with the results of in vivo studies using intravascular administration of cholinomimetic drugs in different animal species.

Besides the presence of muscarinic receptors the results of our experiments also show that nicotinic receptors play a part in the vasodilator response to low concentration of carbachol as hexamethonium \((10^{-3}M)\) proved to prevent the vasodilatation by carbachol.

The possible involvement of nicotinic receptors in the regulation of the cerebrovascular tone has already been demonstrated in vitro by Edvinsson et al. Acetylcholine was found to affect the cerebrovascular tone through presynaptic inhibition of vascular sympathetic discharge and this effect was mediated by nicotinic receptors. This is in contrast with other vascular beds, where a similar effect is mediated by muscarinic receptors.

Moreover it has been demonstrated that the fall in CBF as well as the pial artery constriction caused by sympathetic stimulation can be reduced by carbachol (or acetylcholine) and that this effect is not affected by atropine; it may therefore be concluded that it is caused by a nonmuscarinic mechanism. Provided there is a resting sympathetic tone in the vascular bed under study, the inhibition of the release of noradrenaline from the vascular sympathetic nerve endings may contribute to the increase in CBF caused by carbachol.

In addition to a direct interaction with vascular receptor sites, cholinomimetic drugs can affect the cerebral blood flow in an indirect way also. This is possible on account of the coupling which normally exists between function and blood flow. In the striatum (caudate-putamen) the greater part of the cholinergic innervation is supposed to be intrinsic and predominantly facilitating with respect to striatal cells. Cholinergic mechanisms in the central nervous system involve both muscarinic and nicotinic receptors. In the brain as a whole more muscarinic than nicotinic receptors seem to be present and they are especially numerous in the caudate nucleus. It may therefore be assumed that superfusion of the caudate nuclei with artificial CSF containing carbachol excites striatal cells mainly through the activation of muscarinic receptors. Coupling between function and blood flow then would induce vasodilatation. The fact, however, that the administration of cholinomimetic drugs increases CBF even after inhibition of the neuronal activity by means of GABA or MgSO₄ indicates that such an indirect mechanism is not necessarily involved.

In summary, our experiments show that superfusion of the caudate nucleus with solutions containing lower concentrations of carbachol causes vasodilatation whereas high unphysiological concentrations of carbachol cause vasoconstriction. Both muscarinic and nicotinic receptors are active on the vascular bed under study.

References

Treatment of Acute Focal Cerebral Ischemia With Propranolol

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SUMMARY Propranolol's potential as a protective agent against tissue injury has been noted in experimental myocardial, cerebral, and early acute focal cerebral ischemia. The purpose of the present investigation was to study further the effects of racemic (d,l) propranolol on blood-brain barrier permeability, morphological changes, cortical electrical activity, and regional cerebral blood flow (rCBF) in experimental focal cerebral ischemia. Thirty adult cats, anesthetized with nitrous oxide, underwent 6 hours of right middle cerebral artery (MCA) occlusion. Fifteen cats were untreated. Fifteen cats were given a continuous infusion of racemic propranolol (1 mg/kg/hr) for 7 hours beginning 1 hour before MCA occlusion and a 4 mg/kg bolus immediately before occlusion, both directly into the right carotid artery. Right Sylvian rCBF did not differ significantly between the untreated and treated groups. Carbon filling defects and vital dye (i.e., Evans blue and fluorescein) extravasation were less severe in the propranolol treated animals. Light microscopic findings demonstrated no difference in infarct size between the two groups. The findings suggest that at doses given, racemic propranolol does not exert a protective effect upon cerebral tissue subjected to 6 hours of incomplete ischemia.

Stroke Vol 15, No 3, May-June 1984

486


34. PROPRANOLOL’S POTENTIAL as a protective agent against tissue injury has been reported in experimental myocardial, cerebral, and renal ischemia. Racemic propranolol and its d-isomer also have been shown to favorably modify infarct size in cats subjected to 3 hours of middle cerebral artery (MCA) occlusion. This effect was also associated with a trend toward reduction of blood-brain barrier permeability to vital dye extravasation and less severe cortical and subcortical carbon filling perfusion defects; however, there was no significant difference in regional cerebral blood flow (rCBF) changes between untreated and treated cats. The purpose of the present investigation was to determine if racemic (d,l) propranolol would show the same beneficial effect in cats subjected to 6 hours of incomplete ischemia.

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Received July 6, 1983; revision #1 accepted November 3, 1983.

acutely focal cerebral ischemia produced by MCA occlusion.

Methodology

Animal Preparation

Thirty adult cats were lightly anesthetized with a 75% nitrous oxide and 25% oxygen mixture. Tubocurarine chloride (1 mg/kg) was intravenously administered, an immediate tracheotomy performed, and mechanical ventilation instituted. The right femoral artery and vein were cannulated. Tubocurarine chloride (0.15 mg/ml of normal saline) was administered through an IMED 922 infusion pump (IMED Corp., 9925 Carroll Canyon Rd., San Diego, CA 92131) throughout each experiment. One dose of atropine (0.6 mg/kg) was given subcutaneously. Needle electrodes were placed subcutaneously for continuous EKG monitoring. Mean arterial blood pressure was monitored continuously. A heating pad was placed over the trunk to maintain core temperature at 37°C and was monitored continuously by rectal thermometer. Arterial
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Stroke. 1984;15:481-486
doi: 10.1161/01.STR.15.3.481

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