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^{-5}M) caused vasodilatation whereas high concentrations (10^{-3}M) caused local vasoconstriction. The increase in the local blood flow caused by the low carbachol concentrations was reduced by both atropine (10^{-5}M) and hexamethonium (10^{-3}M). The fall in CBF observed with the high carbachol concentration was prevented by atropine (10^{-5}M).

It may be concluded that low, physiologically more meaningful, carbachol concentrations cause a local vasodilatation due to interaction with both muscarinic and nicotinic receptors.

Methods

Cats of either sex weighing approximately 3 kg were anesthetized with ether followed by an intravenous dose of sodium-thiopental (Pentothal®) (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 measured and the animals were kept in steady state normocapnia (PaCO₂ 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 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 A₁; L 4, H } according to the stereotaxic atlas of Snider and Niemer30). 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.25

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 10⁻⁴ (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 10⁻³M as well as 10⁻⁴M 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 10⁻¹M (n = 5) was added to the perfusion fluid on the experimental side. Low (10⁻³M) and high (10⁻¹M) 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 10⁻⁵M (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.31

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 10⁻³M 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 10⁻⁵M carbachol similarly produced vasodilatation, but the effect was much less 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
experiment size decreased significantly (30%). The mean values of the blood flow during the control period (t = -90 to t = 0) varied between 107 and 99 ml-min⁻¹·100 g⁻¹ on the control side and between 129 and 114 ml-min⁻¹·100 g⁻¹ on the experimental side. After 135 min blood flow dropped to 72 ± 2 ml-min⁻¹·100 g⁻¹ on the experimental side whereas on the control side only a slight decrease was observed. The resulting blood flow ratio (R = E/C) decreased from 1.15 ± 0.08 at t = 0 to 0.83 ± 0.10 at t = 135 (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 animals of which the mean arterial blood pressure remained higher than 100 mm Hg throughout the experiment.

Effects of Atropine and Hexamethonium on the Response to Carbachol

In a second series of experiments the effect of carbachol was studied in the presence of acetylcholine receptor antagonists. After bilateral addition of antagonists to the perfusate, 10⁻⁵ M carbachol was added to the side initially showing the lowest blood flow. This was done in order to detect any possible vasodilatation more easily.

When both lateral ventricles were perfused with artificial CSF containing atropine (10⁻⁵ M) 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 84 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).

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 (10⁻³ M), the blood flow in the caudate nuclei varied between 113 and 105 ml-min⁻¹·100 g⁻¹ on the control side and between 111 and 101 ml-min⁻¹·100 g⁻¹ on the experimental side. After addition of carbachol (10⁻³ M) blood flow remained practically unchanged on both sides. The blood flow ratio (R = E/C) was 0.99 ± 0.16 at t = 0 and 0.95 ± 0.21 at t = 135 (n = 5) (fig. 2B).

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 10⁻³ M whereas a lower (10⁻⁵ M) or a higher (10⁻² 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-
tylcholine (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 epiarachnoid irrigation, the mean concentration of exogenous histamine in the first mm of the brain subjacent to the irrigating solution was only approximately 15% of the concentration of histamine in the irrigation fluid.

Experiments in which very large doses of carbachol ($10^{-3}$M) 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 in vitro preparations: vasodilatation of isolated segments of cerebral arteries with low concentrations (up to $10^{-4}$M) of acetylcholine and vasoconstriction with high concentrations (> $10^{-3}$M). 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 ($10^{-5}$M) clearly impedes the dilatation caused by carbachol ($10^{-3}$M) as well as the vasoconstriction caused by carbachol ($10^{-3}$M). When administered intraventricularly after treatment with atropine, carbachol ($10^{-3}$M) only produced a small and insignificant vasodilatation.

Our experiments thus show that muscarinic recep-
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.15

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.16, 22, 23 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.39 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

JUDITH A. CAPRARO, M.D., D. PETER REEDY, M.D.,* JOHN P. LATCHAW, M.D.,* ROBERT M. SLUGG, B.A.,*
NICHOLAS T. STOWE, PH.D.,† RONALD P. LESSER, M.D., and JOHN R. LITTLE, M.D.*

SUMMARY Propranolol's potential as a protective agent against tissue injury has been noted in experimental myocardial, renal 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 significantly differ in the treated and untreated 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.

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