Effects of Dobutamine on Brain Surface Microvessels in Rats

Shingo KAWAMURA and Nobuyuki YASUI

Department of Surgical Neurology, Research Institute for Brain and Blood Vessels-AKITA, Akita

Abstract

The effects of dobutamine on the diameters of rat pial vessels were investigated in vivo using a closed cranial window technique. Dobutamine (10^-7 to 10^-3 M) was dissolved in artificial cerebrospinal fluid (CSF). Arterioles (17-78 µm in diameter) and venules (20-97 µm in diameter) were observed through the cranial window over the left parietal cortex. Superfusion of the brain surface with only artificial CSF had no effect on vessel diameter. Dobutamine, even at a high concentration of 10^-4 M, did not induce significant diameter changes in the pial vessels, compared with control animals. The arterioles showed marked dilatation (+73%) during superfusion with 10^-3 M dobutamine (p < 0.01 vs. control). The venules were also dilated (+12%), although the increased diameter was not statistically different from controls. Therefore, dobutamine did not induce a dose-dependent dilation. The results strongly suggest that dobutamine at clinical dosages does not have a direct vasomotor effect on brain microvessels.

Key words: dobutamine, brain microcirculation, closed cranial window, intravital fluorescence microscopy, rat

Introduction

Cerebral vasospasm following aneurysm rupture can cause delayed ischemic neurological deficits, and is one of the most important determinants of morbidity and mortality in patients with subarachnoid hemorrhage. Various pharmacological agents, including catecholamines, have been used for the medical management of vasospasm. Our hospital uses dobutamine, a dopamine analog with potent inotropic effects, like those of other therapeutic regimens. The rationale for the use of dobutamine is that increased cardiac output will result in increased cerebral blood flow in ischemic brain tissue, assuming that normovolemic or hypervolemic conditions are maintained to prevent dehydration. We do not believe that the drug acts directly on cerebral blood vessels, thereby affecting cerebral blood flow. However, the absence of direct effects of dobutamine on cerebral blood vessels has not been proved in vivo.

The present study investigated the in vivo effects of dobutamine on brain surface microvessels in rats using a closed cranial window technique and intravital fluorescence microscopy. Dobutamine was applied extravascularly to avoid systemic effects from the drug.

Materials and Methods

I. General preparation

Thirteen male Sprague-Dawley rats (Charles River Japan, Inc., Atsugi, Kanagawa), weighing 250-280 g, were used. Anesthesia was induced by 4% halothane, which was reduced to 1.5% during catheter insertion into a tail artery and femoral veins. Following tracheotomy, α-chloralose (initial dose 80 mg/kg i.v.) was administered, and halothane was discontinued. Supplemental α-chloralose (15 mg/kg/hr i.v.) was continuously given to maintain anesthesia. The animals were immobilized with pancuronium bromide (2 mg/kg/hr i.v.), and artificially ventilated with room air and supplementary oxygen. Arterial blood was collected for blood-gas analyses and hematocrit measurements. Mean arterial blood pressure (MABP) in the tail artery and intracranial pressure (ICP) under the cranial window were recorded continuously. Fluid and drugs were administered into the left femoral vein. Na^+/-fluorescein, used as a blood-brain barrier and vessel marker, was administered into the right femoral vein. Rectal temperature was maintained at 37.5°C by heating.

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II. Cranial window

The procedures of the cranial window will be mentioned briefly. A window was made over the left parietal brain using a dental drill, and a thin bone layer over the dura was left intact. Three catheters (PE 50) were attached to the window for superfusion of the brain surface and for ICP measurements. These were fixed in dental cement. A plexiglass funnel was fixed to the skin above the window and filled with paraffin oil. Under the oil, the remaining bone layer of the window was removed, and the dura was opened. Following infusion of a small amount of artificial cerebrospinal fluid (CSF) over the brain, the window was closed by a glass cover (13 mm diameter) with cyanoacrylate. Finally, the funnel was removed, and the distal end of the outflow catheter of the window was adjusted to a height that maintained an ICP of 5 mmHg.

III. Artificial CSF

During and after a control period of at least 30 minutes, the brain surface was superfused with artificial CSF at 5 ml/hr. The artificial CSF was composed of the following: glucose 3.3 mM, Na+ 158 mM, K+ 3.2 mM, Ca2+ 1.5 mM, Cl− 142 mM, Mg2+ 1.33 mM, and HCO3− 24.5 mM, yielding an osmolality of 307 mosm/l. A mixture of humidified CO2 (6%), O2 (10%), and N2 (84%) was passed through the artificial CSF before superfusion at 37.5°C, yielding a pH of 7.32 ± 0.01 (range 7.30-7.36), a PCO₂ of 46.1 ± 1.1 mmHg (42.2-49.4 mmHg), and a PO₂ of 86 ± 2 mmHg (80-92 mmHg). The pH of the solutions showed no changes after the addition of dobutamine.

IV. Intravital fluorescence microscopy

Intravital fluorescence microscopy was performed using a fluorescence microscope system.6) Epi-illumination of the brain surface was made only during observation (about 10 min for each observation). A 100 W high pressure mercury lamp was used as the light source. Light intensity was reduced through two sets of ND25 filters. A band-pass filter was used to obtain an excitation source with a wavelength of 455-490 nm. The fluorescence emission of the brain surface after i.v. injection of 2% Na+–fluorescein was studied. Wavelengths below 515 nm were excluded during both visual observation and photography. Dye solution (10 μl) was administered for a total of 0.36–0.52 ml during each experiment. A 20× objective lens was used to measure internal diameters of pial vessels, and images were recorded on videotape through a video timer. A low-light TV camera (C2741; Hamamatsu Photonics, Hamamatsu, Shizuoka) and a TV-monitor provided the final magnification (600×). Extravasation of Na+–fluorescein was not observed during the entire observation period, demonstrating that the blood-brain barrier function was normal.

V. Measurements of vessel diameters

Vessel diameters were measured off-line from hand-traced drawings of the vascular network on the brain surface from microphotographs taken through a 2× objective lens. The diameters of randomly selected arterioles and venules were manually measured on the TV monitor using sliding calipers (resolution on the TV monitor 0.01 mm). All measured locations were marked on the drawings to easily identify the exact site of each vessel. A total of 122 arterioles and 77 venules were studied in both drug-treated and control animals.

VI. Experimental protocol

Dobutamine, dissolved in artificial CSF, was serially diluted. Five different concentrations (10−7–10−3 M) were prepared prior to use. Five control animals were used to study the effects of continuous superfusion of pial vessels with artificial CSF. The vessels were observed six times at 30-minute intervals, and the sequential changes in the diameters of 39 arterioles (mean ± SD 42 ± 13 μm, range 17–63 μm) and 26 venules (mean ± SD 50 ± 19 μm, range 20–95 μm) were evaluated. In the remaining eight animals, the brain surface was superfused with the dobutamine solution, and 83 arterioles (mean ± SD 42 ± 15 μm, range 20–78 μm) and 51 venules (mean ± SD 44 ± 18 μm, range 22–97 μm) were evaluated. The test solutions were used in increasing concentrations, and exposure of the cortical surface to any given concentration lasted for 30 minutes.

VII. Statistical analysis

Values are indicated as mean ± SD. Inter- and intragroup comparisons of both physiological data and changes in vessel diameters were performed using Student’s unpaired t-test and Dunnett’s multiple comparison, respectively. Initial and final hematocrits were compared using the paired t-test. A p level of < 0.05 was considered significant.

Results

I. Physiological data

The blood-gas status was stable throughout all experiments in all animals: PaCO₂ was 37.4 ± 1.4 mmHg (range 35.1–41.0 mmHg), PaO₂ was 116 ± 9 mmHg (96–145 mmHg), and pH was 7.43 ± 0.02 (7.37-7.49). The initial hematocrit (43 ± 2%) showed no significant difference to the final hematocrit (42
± 3%). Heart rate and MABP are shown in Fig. 1. Both parameters were stable in the control animals. The heart rate of the dobutamine-treated animals following the superfusion of 10⁻³ M solution increased by about 25 beats/min (p < 0.01 compared with the resting condition), but was not statistically different from the control group (p < 0.07). At the same time, the MABP of the treated animals tended to decrease although this was not significant (p < 0.20 vs. resting condition).

II. Vessel diameter
Changes in the diameter of the pial vessels are shown in Figs. 2 and 3. Control vessels showed no significant diameter changes. The arterioles in the dobutamine-treated animals were dilated by +12% and +73% during 10⁻⁴ M and 10⁻³ M dobutamine superfusion, respectively. The latter diameter was significantly different from the control value (p < 0.01). The venules in the dobutamine-treated animals were also dilated by +12% during 10⁻³ M dobutamine superfusion, although this diameter was not statistically different from the control value.
Discussion

The present study indicated that dobutamine, even at a high concentration (10⁻⁴ M), did not induce significant diameter changes in rat pial vessels compared with control animals. An extremely high concentration of dobutamine (10⁻³ M) did cause marked dilation in arterioles. Dobutamine did not induce a dose-dependent dilation, and the results strongly suggest that dobutamine does not have a direct vasomotor effect on the brain surface microvessels. We used the drug concentrations of 10⁻¹-10⁻³ M because previous studies on monoamines used these concentrations, allowing comparison of the present and previous results. The effective plasma concentration of dobutamine to increase cardiac output in humans is greater than 10 ng/ml, and the half life of the plasma concentration is 3-4 minutes (unpublished data from Shionogi & Co., Ltd., Osaka).

Dobutamine is a sympathomimetic catecholamine, and was developed in an effort to find an agent that had the inotropic properties of drugs such as norepinephrine, isoproterenol, and dopamine, but without significant effects on vasomotor tone or myocardial automaticity. These features make dobutamine particularly suitable for treating patients with low output cardiac failure.

The most prominent effect of dobutamine is a stimulatory effect on cardiac β₁-receptors. Dobutamine can induce a marked increase in cardiac contractility, resulting in an increase in cardiac output, and also increases the heart rate to some degree, but not markedly. In the peripheral vasculature, dobutamine has minor stimulatory effects on both β₂ and α-receptors; β₂-adrenergic dilator action being greater than the α-adrenergic constrictor action. Our present study shows that the heart rate increased with concomitant decrease of blood pressure when 10⁻³ M dobutamine was used (Fig. 1). Apparently dobutamine in the artificial CSF had entered the systemic circulation, probably by absorption into the venous circulation (through the arachnoid villi) because of the extremely high concentration used. A similar phenomenon was observed when ICP was elevated by 5-mmHg steps to exceed 25 mmHg.

Cerebral blood vessels may have both α- and β-receptors. The β₁-receptor may be dominant, and may dilate cerebral vessels. There is evidence suggesting the existence of the β₂-receptor, although its action is unclear. The in vivo effects of dobutamine on cerebral vessels have not been investigated previously, but in vitro administration to cerebral arterial strips did not cause any response. These results are consistent with the present study. Dobutamine apparently does not act on the β₁-receptors on cerebral vessels, nor directly on the specific dopaminergic receptors on cerebral vessels.

Systemically administered catecholamines are believed to have only minimal effects on cerebral tissue perfusion even when infused directly into the internal carotid artery, possibly due to the blood-brain barrier mechanisms for monoamines. An autoradiographic study has indicated that neither intravenous ¹⁴C-dobutamine nor dopamine can penetrate the blood-brain barrier. We administered dobutamine extravascularly in the present study, and observed a marked arteriolar dilation during superfusion with 10⁻³ M dobutamine (Fig. 2). This dilation may have been secondary to absorption of the drug into the systemic circulation. Cardiac output may increase at the same time, but we did not measure the blood concentration of dobutamine, so the mechanisms causing the observed vessel dilatation are unclear. In addition, the relationship between augmentation in the cardiac output and changes in the cerebral blood flow has not been established.

Extravascularly administered dobutamine (10⁻⁷ to 10⁻⁴ M) did not change the diameters of pial vessels compared to a control group as observed through a cranial window in rats. The drug in a clinical dosage is unlikely to act directly on cerebral vessels.

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References

Commentary

Kawamura and Yasui have, in this elegant series of pial window experiments, demonstrated well that the inotropic drug dobutamine has little or no direct effect on the cerebral resistance vessels. At "physiological" or therapeutic concentrations these vessels underwent no change in diameter, and the dilatation seen at the highest concentrations was, as postulated, most likely due to systemic effects following absorption from the CSF.

Dobutamine is used in many centers as part of the "Triple-H" management of cerebral vasospasm, and this demonstration of the absence of any direct effect, particularly no vasoconstriction, is reassuring for this treatment.

Nicholas W. C. DORSCH, F.R.C.S., F.R.A.C.S.
Department of Surgery
Westmead Hospital
Sydney, Australia

This in vivo experimental study investigated the effects of topically applied dobutamine on the diameters of rat pial vessels using a closed cranial window technique. The authors concluded that dobutamine, even at a high concentration (10^{-4} M), did not induce significant diameter changes in rat pial vessels.

This is a well designed experimental work providing some insight into the clinical use of dobutamine for management of cerebral vasospasm following aneurysm rupture which can lead to delayed ischemic neurological deficits.

Kyu Chang LEE, M.D.
Department of Neurosurgery
Yonsei University College of Medicine
Seoul, R.O.K.

DOBUTAMINE AND PIAL VESSELS

Dobutamine, a synthetic catecholamine, has been commonly used in patients who underwent surgical obliteration of a ruptured aneurysm in the acute stage of subarachnoid hemorrhage to prevent cerebral ischemia due to cerebral vasospasm. Dobutamine has been thought to have a potent β_{1}-adrenergic action on cardiac muscles, resulting in the increase in cardiac output and cerebral perfusion pressure. β_{1}-Receptors predominate on cardiac muscles, but are also found in smooth muscles of cerebral vessels, so cerebral vasodilatation can be produced by activation of β_{1}-receptors of the vessel walls.

The authors investigated the effect of dobutamine on brain surface microvessels by fluorescence microscopic observation through a cranial window, to clarify whether the drug acts directly on cerebral microvessels. Dobutamine, when superfused over the cerebral hemisphere in the concentration of 10^{-7}–10^{-4}

Address reprint requests to: S. Kawamura, M.D., Department of Surgical Neurology, Research Institute for Brain and Blood Vessels-AKITA, 6–10 Senshukubota-machi, Akita 010–0874, Japan.
M, had no effect on diameters of cerebral arterioles and venules, though significant dilatation of microvessels was observed in the concentration of $10^{-3}$ M. They conclude that dobutamine at clinical dosages does not have a direct vasomotor effect on cerebral microvessels, and dilatation of microvessels observed in extremely high concentration ($10^{-3}$ M) is probably due to an absorption in the systemic circulation.

These results suggest that intravenous dobutamine in the clinical dosage may increase cerebral blood flow, not by acting on $\beta_1$-receptors of cerebral vessels, but by acting on the receptors of cardiac muscles. The results also indicate that cardiovascular parameters such as cardiac output and circulatory blood volume should be monitored whenever dobutamine is administered to prevent cerebral ischemia in patients with cerebral vasospasm.

In this experiment, the dilatation of microvessels was produced by application of a high concentration ($10^{-3}$ M) of dobutamine. The cause of the vascular dilatation remains obscure, though both the decrease of MABP and increase of heart rate may indicate an inflow of the drug into the systemic circulation. Measurement of the plasma concentration of the drug would be needed to explain the pharmacological properties.

Takashi OHMOTO, M.D.
Department of Neurological Surgery
Okayama University Medical School
Okayama, Japan

Many therapeutic methods are applied to the treatment or the prevention of vasospasm following aneurysmal subarachnoid hemorrhage (ref. 4 of this article).21 Hyperdynamic therapy using the beta-agonist dobutamine is one of the strongest ones. Dobutamine is used not only for the treatment of vasospasm in patients with ruptured aneurysm, but also for severely deteriorated cardiac insufficiency following coronary, renal, hepatic diseases, or for acute respiratory distress syndrome. There are many reports of dobutamine in both clinical and experimental studies, but few papers have investigated the reactive properties of dobutamine in cerebral arterial (or venous) vessels. It is well known that dobutamine increases the cardiac output but affects little or decreases slightly the resistance of the peripheral vessels. And, as mentioned by one of the authors of this paper (ref. 4), cerebral blood flow increases significantly following dobutamine administration in the hyperdynamic therapy of patients with cerebral vasospasm. However, we have had no information whether dobutamine works directly to dilate the cerebral vessels or not. In this paper, authors showed clearly that dobutamine does not have a direct vasomotor effect.

We can agree with their conclusion, although they did not check the blood concentration of dobutamine in their experimental rats. In recent reports, dopexamine, a new synthetic catecholamine for intravenous use is compared to dobutamine or dopamine.1,3,4 Dopexamine has not been approved in Japan. It seems to have interesting vasodilator properties, with marked intrinsic agonist activity at beta-2 adrenoreceptors and a lesser agonist activity at dopaminergic receptors. This new drug has specific activity at dopaminergic receptors for increasing cerebral, myocardial, splanchnic, and renal blood flows.

References


Shin UEDA, M.D.
Department of Neurological Surgery
Center Clinic for the Diseases of CNS
Fukuoka Kieikai Hospital
Fukuoka, Japan

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