Effects of Sympathetic Nerves on Cerebral Vessels during Acute, Moderate Increases in Arterial Pressure in Dogs and Cats

DAVID W. BUSIJA, DONALD D. HEISTAD, AND MELVIN L. MARCUS

SUMMARY We studied: (1) the effects of an abrupt, moderate increase in arterial pressure on total and regional cerebral blood flow (CBF) and (2) whether sympathetic stimulation attenuates the transient hyperemia that occurs during a sudden increase in pressure within the physiological range of pressure. Abrupt increases in arterial pressure were produced by occlusion of the descending aorta. Cerebral blood flow was determined in dogs and cats using radioactive microspheres. In dogs, blood flow to all regions of the brain increased by 35–55% at the onset of hypertension and returned to normal by 60 seconds. Electrical stimulation of sympathetic nerves did not attenuate the transient rise in CBF in dogs. In cats, blood flow increased by 40–60% in cerebrum (cortical grey matter), cerebellum, and brainstem at the onset of hypertension and was still moderately elevated after 2.5 minutes. Electrical stimulation of sympathetic nerves in cats attenuated the initial rise in CBF. At the onset of hypertension in cats, the increase in blood flow to the unstimulated cerebrum was 42% greater than on the stimulated side. Blood flow to cortical grey matter was 71% and 65% greater on the unstimulated side than on the stimulated side at the onset and after 20 seconds of hypertension, respectively. We conclude that an abrupt, moderate increase in arterial pressure within the physiological range produces a transient increase in CBF and, furthermore, that stimulation of sympathetic nerves attenuates the increase in flow in cats. Circ Res 46: 696–702, 1980

RECENT studies suggest that sympathetic nerves may have important effects on cerebral vessels during severe hypertension (Bill and Linder, 1976; Heistad et al., 1978). When mean arterial pressure exceeds approximately 150–180 mm Hg, the autoregulatory capacity of cerebral vessels is exceeded, cerebral blood flow (CBF) increases, and the blood-brain barrier is disrupted. Stimulation of sympathetic nerves attenuates the increase in flow during severe hypertension and reduces disruption of the blood-brain barrier (Heistad and Marcus, 1979).

Although previous studies suggest that sympathetic nerves may have an important protective effect on cerebral vessels during extreme hypertension, an important role of neural control has not been demonstrated within the physiological range of pressure (Heistad and Marcus, 1978). Sympathetic stimulation at normal arterial pressure appears to have little or no effect on CBF in dogs or cats. In these experiments, we have examined effects of sympathetic nerves on CBF during abrupt increases in arterial pressure within the physiological range. We tested the hypothesis that an abrupt increase in arterial pressure might produce a transient increase in CBF, and that sympathetic stimulation might attenuate this hyperemia.

In initial studies, we used a Doppler velocity probe, placed over a large pial artery, to serve as a guide for the appropriate time to inject microspheres to measure CBF. We studied cats, as well as dogs, because recent studies suggest that cats are more responsive than dogs to sympathetic stimulation during sustained severe hypertension (Heistad et al., 1978).

Methods

Seventeen mongrel dogs (14–34 kg) and seven cats (3.4–4.0 kg) were used for these experiments. Dogs were anesthetized with intravenous chloralose (50 mg/kg) and urethane (500 mg/kg). Cats were given sodium methohexital (30 mg/kg) intraperitoneally for initial anesthesia and intravenous chloralose (50 mg/kg) as needed during the experiments. The animals were intubated and ventilated with air and supplemental oxygen. Heparin (500 U/kg, iv) and decamethonium bromide (0.3 mg/kg, iv) were given for anticoagulation and skeletal muscle paralysis, respectively. Arterial blood gases and pH were measured frequently and maintained at normal levels by adjusting the ventilatory rate or by injection of small amounts of sodium bicarbonate.

Arterial pressure was raised by either total or partial occlusion of the aorta. This was done in dogs by suddenly inflating a balloon in the thoracic aorta or by tightening a ligature around the aorta. In
most dogs, arterial pressure decreased toward control level for 5-15 seconds after an initial rise. To sustain the desired level of hypertension during this period, the distal cut end of the ansa subclavia was stimulated (14-20 V, 4-20 Hz and 3 msec) as needed. Stimulation of the ansa increased arterial pressure and heart rate but did not alter the size of the pupil. Arterial pressure was raised in cats by occluding the aorta with a ligature immediately caudal to the diaphragm. In both cats and dogs, the vagosympathetic trunks were resected at the midcervical level to minimize bradycardia during hypertension. With occlusions lasting longer than 60 seconds, ventilatory rate was adjusted to maintain normocapnic levels during hypertension. In dogs, end-tidal CO₂ was continuously monitored using an infrared CO₂ analyzer (model LB-2, Beckman Instruments).

**Measurement of CBF**

After a left thoracotomy, one or two cannulas were placed in the left atrium for injection of microspheres. The two atrial catheters allowed the injection of two differently labeled microspheres within 10 or 20 seconds of each other. In dogs, polyethylene catheters were inserted into the brachial arteries for withdrawal of reference blood samples and, in the internal thoracic and omocervical arteries, for measurement of blood pressure and blood sampling. In cats, polyethylene catheters were inserted into the axillary arteries for withdrawal of reference blood samples. Blood pressure was monitored by a catheter advanced into the thoracic aorta via the femoral artery. Arterial blood samples also were withdrawn through this catheter.

Microspheres, 15 μm in diameter, labeled with ^46Sc, ^95Nb, ^85Sr, ^141Ce, or ^125I, were used for measurement of CBF. Before each injection, the vial containing the microspheres, which were suspended in 10% dextran, was shaken vigorously for several minutes on a Vortex mixer. In dogs, 2-9 million microspheres were injected for each flow determination, and in cats, 0.6-4.5 million microspheres were injected each time. During normotension and steady state hypertension, the microspheres were injected slowly over a 15- to 20-second period and were flushed with saline. At the onset of hypertension, and after 10 or 20 seconds of hypertension, the microspheres were injected as a bolus. Beginning 30 seconds prior to injection of microspheres and continuing for 90 seconds afterwards, two reference arterial blood samples were taken. Using either technique of injection, we determined that the differences in radioactivity contained in simultaneous reference blood samples were small (<4.5%), indicating that mixing of spheres was excellent during bolus injection and slow injection of microspheres.

After each study, the animal was killed with intravenous KCl, and the brain was removed and dissected by region and tissue. Brain samples were classified as right and left cerebrum, cerebellum, brainstem (thalamus-midbrain, pons, and medulla), cerebral white matter (corpus callosum, centrum ovale, and optic chiasm), cortical grey matter, and caudate nucleus. Samples of temporalis muscle from the right and left sides also were taken. The brain and muscle samples weighed from 0.1 to 8 g.

After the tissue samples had been weighed, they were placed in plastic test tubes and counted in a 3-inch well-type γ counter. Blood samples were divided into aliquots so that counting geometry was similar to the tissue samples. The energy windows used were: ^46Sc, 800-1500 keV; ^95Nb, 650-800 keV; ^85Sr, 400-600 keV; ^141Ce, 125-175 keV; and ^125I, 20-50 keV. Output from the γ counter was punched on paper tape for computer processing. Nuclide separation was performed using differential spectroscopy by the method of Rudolph and Heyman (1967).

CBF was calculated from the equation: CBF = C_B × 100 × RBF / C_B, where C_B = cerebral blood flow in ml/min per 100 g, C_B = counts per gram of brain, RBF = reference blood flow (rate of withdrawal of blood samples from reference arteries), and C_T = total counts in the reference arterial blood samples. The counts in the simultaneous reference blood samples were averaged for the calculation of CBF. Temporalis muscle blood flow was determined in a similar manner.

**Measurement of Cerebral Blood Velocity**

Velocity of blood flowing through a large pial artery in dogs was determined by a pulsed-Doppler velocity meter system (Hartley and Cole, 1974). The probe consisted of a 1-mm² piezoelectric crystal mounted at a 45° angle in the tip of a 17-gauge stainless steel needle. The tip of the probe was covered with epoxy to provide a flat end and to improve ultrasound conductance. The crystal was positioned over a branch of the middle cerebral artery through a small burr hole in the skull over the Sylvian fissure. The tip of the probe was lowered so that the surface of the intact dura mater with a stereotaxic instrument and positioned for the optimal Doppler signal. Acoustic gel (Aquasonic 100, Parker Lab Inc.) was spread on the tip of the probe to improve ultrasound conductance.

The crystal was driven by a 20-MHz directional pulsed-Doppler velocity meter. At a constant angle between the crystal and blood vessel, the difference between the transmitted and received frequency (KHz), or Doppler shift, is linearly related to blood velocity. In vitro measurements in our laboratory indicate that the voltage output from the velocity meter is linearly related to velocity over a range of 0-70 cm/sec. We have shown that changes in velocity measured in cerebral vessels with this technique are closely correlated (r = 0.94) with changes in CBF measured with microspheres (Knuepfer et al., 1978). Both phasic (Fig. 1) and mean velocity were recorded.
Temporal Course of Autoregulation

These experiments were designed to examine the temporal course of cerebral autoregulation. In six dogs, pial arterial velocity was measured continuously using the pulsed-Doppler velocity meter. Cerebral blood flow was measured 3 times during the experiment: (1) normotension, (2) at the onset of hypertension (microspheres were injected into the left atrium as the aorta was occluded), and (3) after 60 seconds of hypertension. During this short period of hypertension, no significant change in arterial blood gases or pH occurred.

Differences in CBF and velocity were examined using a randomized block analysis of variance (Neter and Wasserman, 1974). Paired comparisons were made using Duncan's multiple range test (Steel and Torre, 1960).

Sympathetic Stimulation

These experiments were designed to determine whether stimulation of sympathetic nerves attenuates the transient increase in CBF observed at the onset of hypertension. In 11 dogs and seven cats, the preganglionic sympathetic nerves were divided proximal to both superior cervical ganglia. The animals were bled to a resting arterial pressure of 75-95 mm Hg so that arterial pressure after aortic occlusion was still within the “autoregulatory range.” Cerebral blood flow was measured four times during the experiments: (1) normotension, (2) onset of hypertension, (3) 10 seconds later in dogs or 20 seconds later in cats, and (4) after several minutes of hypertension. After the control CBF determination, and 10-20 seconds prior to occlusion of the aorta, one superior cervical ganglion was stimulated at 20 V, 20 Hz, and 3 msec. This level of stimulation, which resulted in maximal dilation of the ipsilateral pupil, was maintained throughout the period of hypertension.

In this study, we injected two differently labeled microspheres 10 or 20 seconds apart to measure transient changes in CBF during hypertension. The injections were made to examine the initial rise in CBF and to estimate the return toward normal. The rationale for the timing is as follows: The peak increase in velocity occurred about 3 seconds after aortic occlusion, and velocity returned to control levels within 10 seconds after the onset of hypertension. We estimated the transit time of blood from the left atrium to the middle cerebral artery in dogs using the pulsed-Doppler velocity meter by injecting a bolus of saline into the left atrium during normotension. As the saline passed the probe placed on the middle cerebral artery, it produced a specific distortion of the output. Transmit time from the left atrium to the middle cerebral artery was 2-4 seconds. Consequently, when microspheres were injected at the time of occlusion, a large fraction of microspheres would pass into the middle cerebral artery within several seconds and thus estimate the peak increase in CBF. All of the microspheres may not lodge in the microvasculature immediately, although most will have passed through large cerebral arteries at this time. Microspheres were injected again after 10 or 20 seconds to document the return of CBF toward normal. Based on our estimate of transit time, there should be little overlap of these two injections.

The percent difference between denervated and stimulated sides during acute hypertension was calculated using the following formula: \( \frac{D - S}{s} \times 100 \), where \( D = \) increase in blood flow from control on the denervated side and \( S = \) increase in blood flow from control on the stimulated side.

Blood flow data were analyzed using randomized block analysis of variance. Significant differences were examined using Duncan’s multiple range test or paired \( t \)-tests with the Bonferonni correction for \( \alpha \) level (Steel and Torre, 1960; Neter and Wasserman, 1974). An \( \alpha \) level of 0.05 was considered significant in all statistical tests.

Results

Temporal Course of Autoregulation in Dogs

When arterial pressure rose from 95 to 130 mm Hg, mean velocity increased rapidly (Fig. 2). Within 10 seconds, however, velocity returned to control levels despite sustained hypertension. The peak increase in velocity occurred 2-4 seconds after aortic occlusion. Peak velocity increased by 64% at the onset of hypertension and returned to control levels by 60 seconds, although arterial pressure remained elevated (Fig. 3). Similarly, blood flow to the cerebrum increased by 48% at the onset of hypertension and returned to control by 60 seconds (Fig. 3). This
CEREBRAL BLOOD FLOW DURING MODERATE HYPERTENSION/Busija et al.

Figure 2  Mean velocity in the middle cerebral artery during an abrupt, moderate increase in arterial pressure.

The difference between velocity and blood flow may reflect the time constraints of the microsphere technique; we compared peak velocity with blood flow measured over several seconds with microspheres. It is likely that peak CBF was higher than measured. Total CBF also increased at the onset of hypertension and returned to control by 60 seconds. Total CBF was: 26 ± 4 ml/min per 100 g during control; 38 ± 5 ml/min per 100 g at the onset of hypertension; and 29 ± 4 ml/min per 100 g at 60 seconds.

CBF was lower in one group of dogs (Fig. 3) than in the other (Table 1). This reduction in resting flow may have been related to the effects of anesthesia or the length of the study. However, cerebral vessels retained their normal responsiveness in both groups of dogs, since they autoregulated quickly during hypertension. There was no difference in blood flow to the cerebrum on the side with the Doppler probe and to the contralateral intact side.

Sympathetic Stimulation in Dogs

At the onset of hypertension, blood flow to all regions of the brain increased by 35-55% in dogs (Table 1). After 10 seconds of hypertension, blood

**Table 1  Sympathetic Stimulation during Moderate Hypertension in Dogs**

<table>
<thead>
<tr>
<th></th>
<th>Normotension</th>
<th>Onset</th>
<th>10 sec</th>
<th>250 sec</th>
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<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>85±2</td>
<td>127±5†</td>
<td>124±6†</td>
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<td>Arterial blood gases</td>
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<td></td>
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<td>Pco₂ (mm Hg)</td>
<td>36±1</td>
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<tr>
<td>pH</td>
<td>7.33±0.02</td>
<td>7.39±0.02</td>
<td></td>
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<tr>
<td>Po₂ (mm Hg)</td>
<td>139±10</td>
<td>169±16†</td>
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<tr>
<td>Cerebrum</td>
<td>39±4</td>
<td>39±4</td>
<td>58±9†</td>
<td>57±8†</td>
<td>48±6†</td>
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<td>53±4</td>
<td>76±9†</td>
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<td>Cortex</td>
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<td>58±6</td>
<td>83±13†</td>
<td>85±12†</td>
<td>69±11‡</td>
</tr>
<tr>
<td>Caudate nucleus</td>
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<td>22±2</td>
<td>34±4†</td>
<td>33±5†</td>
<td>27±5‡</td>
</tr>
<tr>
<td>Cerebral white matter</td>
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<td>40±3</td>
<td>54±6†</td>
<td>54±6†</td>
<td>47±5†</td>
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<td>Brainstem</td>
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<td>52±6</td>
<td>74±10†</td>
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<td>68±8‡</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>7±1</td>
<td>8±1</td>
<td>38±5†</td>
<td>5±2‖</td>
<td>65±13†</td>
</tr>
</tbody>
</table>

* Values are mean ± se in 11 dogs, except for 250 seconds when n = 8. Measurements were obtained in both hemispheres, during sympathetic stimulation to one hemisphere.

† Significantly different from control.
‡ Significantly different from onset.
§ Significantly different from 10 seconds.
‖ Significantly different from other side.
Table 2  Sympathetic Stimulation during Moderate Hypertension in Cats

<table>
<thead>
<tr>
<th></th>
<th>Normotension</th>
<th>Onset</th>
<th>20 sec</th>
<th>150 sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>83±2</td>
<td>133±4†</td>
<td>134±3†</td>
<td>133±3†</td>
</tr>
<tr>
<td>Arterial blood gases</td>
<td></td>
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<td></td>
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<tr>
<td>Pco₂ (mm Hg)</td>
<td>31±1</td>
<td></td>
<td></td>
<td>30±2</td>
</tr>
<tr>
<td>pH</td>
<td>7.42±0.02</td>
<td></td>
<td></td>
<td>7.38±0.01†</td>
</tr>
<tr>
<td>Po₂ (mm Hg)</td>
<td>167±5</td>
<td></td>
<td></td>
<td>166±5</td>
</tr>
<tr>
<td>Regional cerebral blood flow (ml/min per 100 gm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebrum</td>
<td>39±2</td>
<td>66±6†</td>
<td>57±4†</td>
<td>50±5†</td>
</tr>
<tr>
<td>Cortex</td>
<td>75±6</td>
<td>132±8†</td>
<td>102±5†</td>
<td>109±10†</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>42±4</td>
<td>51±7</td>
<td>43±3</td>
<td>54±11</td>
</tr>
<tr>
<td>Cerebral white matter</td>
<td>22±2</td>
<td>25±2</td>
<td>32±4</td>
<td>25±4</td>
</tr>
<tr>
<td>Brainstem</td>
<td>41±4</td>
<td>57±6†</td>
<td>57±6†</td>
<td>54±9†</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>44±2</td>
<td>76±5††</td>
<td>70±4†</td>
<td>67±7†</td>
</tr>
<tr>
<td>Temporalis muscle blood flow (ml/min per 100 gm)</td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

* Values are mean ± SE in seven cats. Measurements were obtained in both hemispheres, during sympathetic stimulation to one hemisphere.
† Significantly different from control.
§ Significantly different from onset.
|| Significantly different from other side.

Sympathetic Stimulation in Cats

In cats, blood flow on the unstimulated side increased by 40-60% in cerebrum, cortical grey matter, cerebellum, and brainstem (Table 2). By 20 seconds, blood flow had begun to return toward control values in these areas, but remained higher than control after 2.5 minutes. Blood flow to cerebral white matter and caudate nucleus did not increase during hypertension.

Sympathetic stimulation attenuated the increase in CBF during sudden hypertension in cats (Fig. 4, Table 2). The increase in flow at the onset of hypertension was 42% greater on the unstimulated cerebrum than on the stimulated side, and in cortical grey matter, the increase was 71% greater on the denervated side. Cortical grey blood flow was still 65% greater on the unstimulated side than the stimulated side after 20 seconds of hypertension. The increase in flow in cerebellum and brainstem during hypertension was unaffected by sympathetic stimulation as expected since there is little innervation from the superior cervical ganglion in these areas.

Blood flow to temporalis muscle was significantly reduced on the stimulated side throughout hypertension (Table 2).

Discussion

These studies demonstrate that abrupt increases in arterial pressure produce transient increases in flow in all areas of the brain and that the autoregulatory response is rapid. The autoregulatory response appeared to be more rapid in dogs than in cats.
Cerebral Blood Flow During Moderate Hypertension/Busija et al.

There are differences in the time course of autoregulation, which may reflect species variation or differences in methods of measuring CBF. These studies suggest that cerebral vessels respond reasonably quickly to increases in arterial pressure.

The present study provides new information on the time course and regional responses of autoregulation in cerebral vessels. There is a large increase in CBF in almost all areas of the brain at the start of hypertension, but the vessels respond quickly so that flow begins to return to normal after 10-20 seconds. In dogs, autoregulation is relatively fast. Blood flow to areas such as the cerebrum is restored to normal within 60 seconds. In cats, autoregulation may be slower except in areas such as cerebral white matter and caudate nucleus which show excellent autoregulation. Since the initial arterial pressure and the magnitude of the increase were similar in both species, the explanation for this apparent difference is not clear.

Responses to Sympathetic Stimulation

We have shown previously that there are major species differences in responsiveness to sympathetic stimulation during normotension and hypertension (Heistad et al., 1978). Although the role of nerves in regulation of CBF continues to be controversial (Purves, 1978; Heistad and Marcus, 1978), it appears that there is little or no decrease in CBF during sympathetic stimulation during normotension in cats and dogs. During sustained severe hypertension, when flow increased 2- to 4-fold, sympathetic stimulation produced a modest attenuation of the increase in flow in dogs and a marked attenuation in cats. The results of the present study also suggest that cats are more responsive than dogs to stimulation of sympathetic nerves during hypertension.

The explanation for this species difference is unclear. Cerebral vessels of both species are richly innervated with sympathetic nerves, and the distribution is largely ipsilateral (Mueller et al., 1977; Nielsen and Owman, 1967). It is interesting that the increase in blood flow is greater in grey matter than white matter during steady state, severe hypertension (Heistad et al., 1978; Heistad and Marcus, 1979) and abrupt, moderate increases in arterial pressure. In addition, the increase in blood flow to cortical grey matter is greater in cats than dogs under these two conditions. Effects of sympathetic stimulation appear to be more pronounced in regions where and in species in which the increase in flow is greatest during hypertension.

It is unclear why stimulation of sympathetic nerves affects CBF during a sudden increase in arterial pressure but not during steady state normotension in cats. We have considered two mechanisms to explain this finding. First, sympathetic stimulation, begun prior to raising arterial pressure, may restrict the passive dilation of cerebral vessels and attenuate the increase in blood flow. Wei et al.
prevents over-perfusion of the brain and disruption of the blood-brain barrier (Heistad and Marcus, 1979). Sympathetic pathways during severe hypertension increase the response of cerebral vessels to serotonin is increased by stretching the vessels. Toda et al. (1978) have shown that the increase in smooth muscle tone may make the walls of large cerebral vessels less distensible. In addition, if large cerebral arteries are constricted during sympathetic stimulation, the increase in pressure would be attenuated in distal vessels. A second possibility is that passive dilation of cerebral vessels at the onset of hypertension increases vascular responsiveness to sympathetic stimulation in a nonspecific manner. Toda et al. (1978) have shown that the response of cerebral vessels to serotonin is increased by stretching the vessels.

**Implications**

Previous studies have shown that stimulation of sympathetic pathways during severe hypertension prevents over-perfusion of the brain and disruption of the blood-brain barrier (Heistad and Marcus, 1979). This study extends the role of sympathetic nerves to the physiological range of arterial pressure. It seems likely that increased sympathetic tone at the onset of a rise in arterial pressure within the physiological range would attenuate an increase in CBF until the autoregulatory process restores blood flow to control levels (Fig. 5). These data suggest that sympathetic nerves may play an important role in the control of CBF during changes in arterial pressure under normal conditions in cats.

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