Effect of Clonidine on the Cerebrovascular System in Cats

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Abstract. The effects of clonidine, a potent α₂ adrenergic agonist and an imidazole/imidazoline receptor agonist, were examined in the cat cerebrovascular system, measuring cerebral oxygen and carbon dioxide tension (BrPo₂, BrPco₂) and arterial blood pressure. Intracarotid injection of clonidine (2 μg/kg) produced a gradual decrease in systemic blood pressure without initial hypertension, while BrPo₂ decreased slightly but significantly. Before and after intracarotid administration of clonidine, cerebrovascular reactivity to carbon dioxide was estimated by changes in BrPo₂ and BrPco₂ during and after 3 min inhalation of 5% CO₂. Clonidine significantly enhanced cerebrovascular reactivity to carbon dioxide. The data suggest that the α₂-adrenoceptor and/or imidazole/imidazoline receptor play an important role in the regulation of cerebral circulation. (Keio J Med 42 (2): 64-69, June 1993)

Key words: cerebral circulation, α₂-adrenoceptor, imidazole/imidazoline receptor, cerebral oxygen tension, CO₂ reactivity

Introduction

α-adrenoceptors of peripheral tissues can be classified into three types, ie postsynaptic α₁-receptor and pre- and postsynaptic α₂-adrenoceptors. In the central nervous system, especially in the brainstem, the existence of α₁ and α₂-adrenoceptors have been also reported. However, only few reports on the role of the α₂-adrenoceptor in the regulation of cerebral hemodynamics have appeared in vivo. Cerebral circulation is controlled mainly by metabolic factors (chemical control) while the sympathetic nervous system (neurogenic control) is considered to play a minor role under normal conditions. Intravenous injection of clonidine, an α₂-agonist and imidazole/imidazoline receptor agonist, causes an initial rise in blood pressure which is followed by a gradual fall. When injected intracisternally, intraventricularly or intravertebrally, clonidine shows only long lasting hypotension. Therefore, in the present study, to minimize the systemic influence of clonidine, we administered a relatively low dose of clonidine by intracarotid injection and examined the cerebrovascular effect of clonidine by continuously measuring cerebral tissue oxygen and carbon dioxide tension (Brain Po₂ = BrPo₂, Brain Pco₂ = BrPco₂) and systemic arterial blood pressure. Furthermore, in order to explore the role of the α₂-adrenoceptor and/or imidazole/imidazoline receptor in the regulation of cerebral hemodynamics, the cerebrovascular reactivity to CO₂ was evaluated before and after clonidine injection.

Methods

Nine cats weighing 2.8–3.5 kg (mean 3.2 kg) were used. Cats were anesthetized with an intraperitoneal injection of α-chloralose (50 mg/kg) and urethane (500 mg/kg) and with 0.5% procaine hydrochloride for local anesthesia. After tracheostomy, ventilation was maintained to keep tidal volume and respiratory rate constant by means of a respirator (Harvard Model 662, Harvard, MA, USA). After immobilization with an intravenous injection of alcuronium chloride, ventilation was started. Abdominal aortic pressure was measured with a pressure transducer (Statham P231DB, Gould, Oxnard, CA, USA) connected to a polyethylene tube inserted into the abdominal aorta through the right femoral artery. The polyethylene tube, inserted retrogradely into the left lingual artery,
was used for the injection of clonidine into the carotid artery. The rectal temperature of cats was kept at 37°C by means of a heating blanket throughout the experiment.

The skull was fixed in an stereotactic head holder (Type SN-1, Narishige, Tokyo, Japan) and the scalp and the dura over the parietal cortex region were removed. Po2 and Pco2 electrodes were attached on the exposed cerebral cortex. The output signal from the Po2 electrode was amplified by a gas analyzer (Beckman Model 160, Beckman Toshiba, Tokyo, Japan). The output signal from the Pco2 electrode was amplified by a gas analyzer (Type SN-1, Narishige, Tokyo, Japan) and the scalp and the dura over the parietal cortex region were removed.

Pco2 electrode

Carbon dioxide was measured by changes in the pH of a weak bicarbonate solution within a Teflon membrane by diffusion of CO2 from outside the membrane. The observed changes in pH are proportional to the unknown Pco2 outside the membrane.

The Pco2 of the pial surface of the cortex was recorded with a pH electrode (Fuji Chemical Industry, Tokyo, Japan) having a flat (McInnes type) surface measuring 3 mm in diameter. A flat Ag-AgCl reference electrode insulated from the glass surface was attached to the electrode by a rubber or plastic ring. This unit was immersed in the Pco2 electrode solution (0.0005 M NaHCO3 + 0.45% NaCl; to each 100 ml of this stock solution was added 1 drop of saturated KCl/AgCl solution). A membrane 12 μ thick was secured around it with silk. The membrane permitted free diffusion of gases but not of ions.

The output of the electrode was recorded with a Model 22 Radiometer pH meter fitted with a 1.5 volt battery and potentiometer for balancing purposes. The output from the meter was stepped down with a resistor acting as a voltage drop and connected to the input terminals of the polygraph.

The electrode was calibrated in a constant temperature gas chamber similar to those used for calibrating the Po2 electrode. The electrical output of the electrode (temperature adjusted to 35°C for brain surface) was then determined after reaching equilibrium with authentic gas mixtures having known CO2 tension. The following gases or mixtures were used: 100% N2 = 0 mmHg; 5% CO2 + 95% N2 = 36 mmHg; 10% CO2 + 90% N2 = 71 mmHg.

The needle of the pH meter was adjusted manually with the balancing device so that all measurements within physiological range could be recorded on the scale. The calibration points were then plotted as a straight line on semi-logarithmic graph paper so that all readings of Pco2 in the physiological range could be estimated from the recorded potentials. The pH meter could thus be used as a direct reading instrument over the whole range of Pco2 encountered. The electrode has a latency within 1–4 seconds, and is stable and accurate.

Clonidine solution (2 μg/kg) was injected into the carotid artery for 3 min. Changes in BrPo2, BrPco2 and blood pressure (BP) were observed for 10 min after the start of the injection. Before and after clonidine injection, inhalation of 5% CO2 in air for 3 min was performed. Cerebrovascular reactivity to CO2 was estimated by changes in BrPo2 and BrPco2 (ΔBrPo2, ΔBrPco2).

Results

Intracarotid injection of clonidine

A typical recording during the injection of clonidine (2μg/kg) into the carotid artery is illustrated in Fig 1. BrPo2 decreased from 24.5 mmHg to 16.0 mmHg after the injection of clonidine. BrPco2 showed no distinct changes during infusion. Blood pressure decreased slightly. These changes in 9 cats are summarized in

Fig 2. BrPo2 continued to decrease during 4–7 min after the start of the injection and was then maintained at a level of about 3–4 mmHg lower than the control values. Changes in BrPco2 values were not statistically significant. Mean arterial blood pressure (MABP) continued to decrease during 1–5 min after the start of the injection and then was maintained at a level of about 4–5 mmHg lower than the control values. These decreases in MABP were slight but statistically significant.

Fig 3. Traces demonstrating effects of CO2 inhalation before and after clonidine infusion on BrPo2, BrPco2 and BP. Before the administration of clonidine, BrPo2 and BrPco2 increased following CO2 inhalation. The same pattern of changes was seen after the administration of clonidine, but the increase in BrPo2 before clonidine (ΔBrPo2 = 12.5 mmHg) was less than that after clonidine (ΔBrPo2 = 18.0 mmHg).
Fig 4 Summarized data demonstrating effects of CO₂ inhalation before and after clonidine infusion on the changes of BrPo₂, BrPco₂ and MABP. Each point represents mean ± SD. N = 9. *P<0.05 and **P<0.01 indicate significant differences between values before and after the administration of clonidine. ΔBrPo₂ became significantly greater after clonidine administration. BrPo₂ at 3min after initiation of CO₂ inhalation was 7.4 ± 4.7mmHg and 12.1 ± 7.3mmHg, before and after clonidine, respectively. BrPco₂ and MABP increased during CO₂ inhalation but there was no statistically significant deference between values before and after clonidine.

Changes in cerebrovascular reactivity to CO₂

A typical recording of CO₂ inhalation before and after clonidine injection is illustrated in Fig 3. Before the administration of clonidine, BrPo₂ and BrPco₂ increased following CO₂ inhalation. The same pattern of changes was seen after the administration of clonidine, but the increase in BrPo₂ before clonidine (ΔBrPo₂ = 12.5 mmHg) was less than that after clonidine (ΔBrPo₂ = 18.0 mmHg). These changes in 9 cats are summarized in Fig 4. ΔBrPo₂ at 3min after initiation of CO₂ inhalation was 7.4 ± 4.7 mmHg and 12.1 ± 7.3 mmHg, before and after clonidine, respectively. BrPo₂ and MABP increased during CO₂ inhalation but there was no statistically significant difference between values before and after clonidine.

Discussion

Clonidine is a potent and highly specific α₂-agonist. It is well known that α₁-adrenoceptors in peripheral vessels contribute to the regulation of peripheral vascular resistance and are of major clinical importance as a site of action of antihypertensive agents. On the other hand, presynaptic α₂-receptors are believed to modulate the release of noradrenaline from sympathetic nerve terminals by a negative feedback mechanism. There are also data suggesting the existence of a postsynaptic α₂-receptor, which can be activated by α₂-agonists to produce vasoconstriction in rats. Concerning the subtypes of α-adrenoceptors in the cerebral arteries, the presence of inhibitory presynaptic α₂-adrenoceptor and vasoconstrictive postsynaptic α₁- and α₂-adrenoceptors has been demonstrated. While it was originally believed that clonidine was an α₂-adrenergic receptor agonist, it is now recognized that this imidazoline also interacts with a distinct receptor class that recognizes the imidazole portion of the molecule's structure. Imidazole/imidazoline receptors play a critical role in the cardiovascular action of clonidine in the brainstem.

A novel small molecule found in brain and other tissues, clonidine-displacing substances (CDS), which binds to imidazole/imidazoline receptors, may be a native ligand of imidazole/imidazoline receptors.

Intravenous injection of clonidine (3–30 μg/kg) induces dose-dependent changes in BP and heart rate; the biphasic change in BP being characterized by an immediate, but transient, increase followed by a gradual fall in BP. In our present study, the intracarotid injection of a relatively low dose of clonidine (2 μg/kg) induced a slight decrease in BP without any initial hypertension. The absence of this initial hypertension is similarly reported in the case of the administration of low dose of clonidine into the cisterna magna, the lateral ventricle and the vertebral artery. The mechanism of the transient hypertension after intravenous injection is assumed to be due to the direct constriction of peripheral vascular smooth muscle mediated by postsynaptic α₂-adrenoceptors. Intracarotid injection of low dose of clonidine should minimize systemic influence, so that transient hypertension would not be observed. The hypotensive effect of clonidine is considered to be the result of a sympathetic central action, or more recently, the result of central hypotensive imidazoline action in the rostral ventrolateral medulla oblongata (RVL). In 1967, clonidine had already been shown to be a strong inhibitor of the vasomotor center in the medulla oblongata.

During the changes of BP, BrPo₂ decreased slightly but significantly. BrPo₂ is determined by the balance between the magnitude of oxygen supply by cerebral blood perfusion and the degree of oxygen consumption due to cerebral energy metabolism. The decrease in BrPo₂ we observed suggests; 1. a decrease in oxygen content in the arterial blood; 2. a decrease in oxygen supply induced by the decreased cerebral blood flow (CBF); and/or 3. an increase in crebral energy metabolism. We did not observe any initial hypertension with intracarotid injection of clonidine, suggesting that the cerebral arteries were supplied with higher concentration of clonidine than the systemic arteries. Accordingly, we speculate that clonidine selectively constricted the cranial vasculature mediated by the postsynaptic α₂-adrenoceptor, which induced a decrease in CBF and
consequently, a decrease in BrPo2.

The cerebrovascular action of CO2 is due to the direct action on the cerebral vessel wall as described by Meyer and Gotoh.23 Arterial CO2 diffuses to the cerebral vessel wall and increases intracellular [H+] of vascular smooth muscle by the action of carbonic anhydrase. The increase of the hydrogen ion concentration is responsible for the cerebral vasodilatation. On the other hand, sympathetic innervation to cerebral vessels is present both morphologically26 and functionally.27 Excitation of the autonomic nervous system by CO2 induces not only peripheral vasoconstriction but also cerebral vasoconstriction. In normal cerebral circulation, direct cerebral vasodilatory action by CO2 (chemical control) overcomes this vasoconstrictory action through the neurogenic mechanism.12

It has been reported that the presynaptic α2-adrenoceptor is present not only in the systemic blood vessels but also in the cerebral vessels.28 Reichl and Walland observed that constriction of pial arteries in response to sympathetic nerve stimulation attenuates after the administration of clonidine in cats and it was assumed that clonidine suppresses neurotransmission in the pial sympathetic nervous system via the presynaptic α2-adrenoceptor.6 Kobari6 also reported that the vasoconstrictive response of the pial arteries during reinfusion of the blood was significantly disturbed after intravenous administration of clonidine in cats. In the present study, clonidine significantly enhanced cerebrovascular reactivity to CO2. This phenomenon is supposed to be the consequence of the inhibition of the neurogenic vasoconstrictory mechanism in cerebral vessels during CO2 inhalation and the enhancement of direct dilatatory response of cerebrovascular smooth muscle to CO2. At present, there is no information about the existence or nonexistence of imidazole/imidazoline receptors in cerebral vessels. However, idazoxan and cirazoline (both are imidazoline drugs) have been recently shown to mediate an inhibition of noradrenaline release in the pulmonary artery and aorta through the activation of presynaptic imidazoline receptors, which suggests that these drugs can act as agonists on these peripheral receptors.15,16 In any case, the present data suggest that α2-adrenoceptors and/or imidazoline/imidazoline receptors play an important role in the regulation of cerebral circulation.

References


