Inhibition by Ketanserin Of Serotonin Induced Cerebral Arteriolar Constriction

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SUMMARY We studied the effects of serotonin on pial arterioles in anesthetized cats equipped with acutely implanted cranial window for the observation of the pial microcirculation. Serotonin topical application caused cerebral arteriolar constriction. Ketanserin, a specific 5-HT₂ inhibitor, completely blocked vascular response of serotonin. Aggregated platelet supernatant was topically applied and caused generalized cerebral arteriolar constriction that could be blocked with ketanserin. We conclude that serotonin causes generalized cerebral arteriolar constriction that is due to the stimulation of 5-HT₂ receptors. Aggregating platelets release serotonin, which mediates the vasoconstrictive action of the supernatant solution.

Methods

Experiments were carried out in cats weighing between 2 and 4.5 kg. The animals were anesthetized with intravenous sodium pentobarbital (30 mg/kg). After completion of a tracheostomy, each cat was ventilated with a positive pressure respirator and received decamethonium bromide, 0.4 mg/kg, iv for skeletal muscle paralysis. End-expiratory CO₂ concentration was monitored with a Beckman infrared CO₂ analyzer and was maintained at a constant level between 30 and 35 mmHg throughout each experiment by adjustment of the tidal volume and respiratory rate, except in instances where it was deliberately changed. Arterial blood pressure was measured with a Statham pressure transducer connected to a cannula introduced into the aorta through the femoral artery. Arterial blood samples were periodically collected for determination of PaCO₂, PaO₂, pH and hematocrit. Blood gas tension and pH were measured with Radiometer electrodes. Hematocrit was measured with a micromethod.

The pial microcirculation of the parietal cortex was visualized via an acutely implanted cranial window, as described in detail previously. The window was equipped with three openings. Two openings served as inlet and outlet for filling the space under the window with artificial cerebrospinal fluid (CSF). The outflow of the window was connected to a plastic tube whose distal end was set at a fixed height to maintain intracranial pressure at 5 mmHg throughout the experiment. In addition, this positive pressure prevented the brain surface from making contact with the glass plate of the window and assured that topically applied solutions completely covered the area of the vessels under observation.

The pial microcirculation on the parietal cortex was observed through the cranial window with a Leitz Uitropak microscope equipped with a Vickers image-splitting device. The cranial window was filled with artificial CSF sharing the same composition as normal CSF for cats.

Venous blood was collected from normal human volunteers by the Richmond Metropolitan Blood Service. The whole blood was mixed with 63 ml of the standard acid citrate dextrose solution (ACD). Platelet rich plasma was removed after centrifugation.
20 min) in a DuPont Sorvall centrifuge. The platelets were resedimented (1000g, 15 min), separated, and then washed with Tyrode's solution at pH 6.35, containing 2mM MgCl\textsubscript{2} but no Ca\textsuperscript{2+}, 3.5 gm/liter bovine serum albumin and 100 mg/liter apyrase. Then the platelets were washed with Tyrode’s solution at pH 6.35, containing 1.4 mM CaCl\textsubscript{2}, 3.5 gm/liter bovine serum albumin, and 100 mg/liter apyrase. An aggregate-free suspension of platelets was obtained. To test the viability of the platelets, 0.3 ml of this suspension was placed in a siliconized test tube and allowed to aggregate with the addition of 0.2 micrograms of epinephrine in an aggregometer. The remainder of the solution was transferred to a non-siliconized glass container and allowed to reach room temperature. Spontaneous aggregation took place. The spontaneously aggregated-platelet supernatant was highly stable as it showed no significant loss of activity. Cat platelets were not used due to the difficulty in harvesting an adequate amount. Platelet rich plasma placed under the cranial window to study in situ aggregation obscured visualization of the vessels and therefore proved unsuitable.

Serotonin hydrochloride was dissolved in artificial CSF at concentrations of 10\textsuperscript{-10} to 10\textsuperscript{-4}M immediately before use. Ketanserin was dissolved in acetone to produce a stock solution of 1.3 mg/ml. From this stock solution the appropriate amount of the drug was diluted in about 10 ml of 0.9% NaCl solution and was administered iv by slow injection.

The experimental design was as follows: After control observations of blood pressure, intracranial pressure and vessel caliber were made, the responsiveness of vessels to change in PaCO\textsubscript{2} was determined by inducing arterial hypocapnia via passive hyperventilation. Only animals in which the pial arterioles constricted by 10–15% of the control diameter following an approximately 50% reduction in PaCO\textsubscript{2} from about 30 mmHg were used for experimentation. One animal was discarded for this reason. Several arterioles covering a wide range of caliber were studied in each animal. The vessels were divided arbitrarily into small arterioles (<100\mu m in diameter) and large arterioles (>100\mu m in diameter), because the responses of vessels of these two size ranges to other interventions are quantitatively different.\textsuperscript{10, 11}

Three series of experiments were carried out. In the first series, we investigated the effect of arteriolar caliber of topical application of serotonin 1 × 10\textsuperscript{-10}, 1 × 10\textsuperscript{-7}, and 1 × 10\textsuperscript{-4}M. Each of these solutions was used to fill the space under the cranial window. Measurements were made between 2 and 4 minutes after application when vessel caliber achieved a new steady state. Then the animals were treated with ketanserin 0.5 mg/kg, iv, and the arteriolar responses to serotonin were determined again 30 minutes later. In the second set of experiments we determined arteriolar responses before and after injecting ketanserin 1 mg/kg. The third series of experiments consisted of adding 0.3 ml of activated platelet supernatant in the space under the cranial window and measuring vessel size for the succeeding three minutes. After the vessels were back to control size, ketanserin 1 mg/kg was injected iv and the effect of activated platelet supernatant was tested again. Serotonin at a concentration of 1 × 10\textsuperscript{-4}M was then injected under the window as a control to test the blocking effect of ketanserin.

The results were analyzed using analysis of variance (PROC GLM).\textsuperscript{12} Least squares means were calculated and compared using student’s t test.

**Results**

The administration of ketanserin caused no significant change in mean arterial blood pressure or in PaCO\textsubscript{2}.

Figures 1 and 2 show the effect of ketanserin on the response of cerebral vessels to topically applied serotonin. Serotonin induced dose dependent constriction of both small and large arterioles. There was no significant difference between the response of small and large vessels. The administration of 0.5 mg/kg of ketanserin caused significant reduction in the vasoconstriction from serotonin. The administration of 1 mg/kg of ketanserin had an even more pronounced effect. At this dose, the vasoconstriction was abolished completely.

Figure 3 shows the response of cerebral arterioles to topical application of the supernatant from activated...
platelets. Before the administration of ketanserin, the supernatant caused constriction of both small and large arterioles. In the large arterioles this response was sustained for the entire period of observation. In the small arterioles, diameter returned to a value insignificantly different from control due to dilation of two vessels in one animal. The vasoconstrictive effect of supernatant was the same immediately after preparation and several days later. After the administration of ketanserin, this vasoconstriction in the small vessels was abolished and it was diminished by 66% in the large vessels.

Discussion

These results support the following important conclusions: 1) Topical application of serotonin by superfusion causes cerebral arteriolar constriction. 2) This vasoconstriction is due to stimulation of 5-HT$_2$ receptors. It is blocked completely by ketanserin. 3) No other vascular effects from the action of serotonin are evident, since its application following pre-treatment with ketanserin causes no significant vascular changes. 4) Aggregating platelets release serotonin, which is the principal stable agent responsible for the vasoconstrictive action of supernatant solution.

Our findings differ from those of Harper and McKenzie who found that microapplication of serotonin caused dilation of small arterioles and constriction of larger vessels. There are several technical differences between their experiments and ours. The most important of these are the following: 1) The type of anesthesia was different. They used chloralose, while we used pentobarbital. 2) They used an open preparation in which the brain surface was covered with mineral oil, while we used a cranial window preparation. 3) They applied serotonin next to the vessel studied by microapplication, while we applied it by superfusion. 4) There were differences in the composition of the artificial CSF used to dissolve serotonin and apply on the brain surface. We do not know which of these differences accounted for the different results.

Our findings show that ketanserin blocked completely the vasoconstrictive effect of serotonin. No vasodilative influences were uncovered following blockade of 5-HT$_2$ receptors by ketanserin. It is clear that, since the constriction of cerebral arterioles caused by serotonin was completely blocked by ketanserin, it must have been mediated by stimulation of 5-HT$_2$ receptors. Such receptors are present in both vascular smooth muscle as well as in brain parenchyma. Since in our preparation, by virtue of the anatomical relationships, the vessels are more accessible to the action of topically applied agents than the brain parenchyma and since stimulation of 5-HT$_2$ receptors of vessels in our
vascular beds causes vasoconstriction, it seems reasonable to conclude that the constriction we observed was due, in large part, to the stimulation of vascular 5-HT2 receptors. On the other hand, accessibility of parenchymal 5-HT2 receptors by agents applied on the brain surface is less certain. We also do not know what the vascular consequences of stimulation of these receptors might be. If they are accessible and their stimulation causes vasoconstriction, we must presume that this action is weak and was overcome by the direct effect of serotonin on the cerebral arterioles. Since brain tissue also contains 5-HT2 receptors, it would appear that these receptors are either not accessible to serotonin as we used it in our experiments, or that their stimulation has no vascular consequences.

We found that supernatant from aggregating platelets caused cerebral arteriolar constriction and that this effect was due to serotonin since it was completely blocked by ketanserin. These results suggest that the main stable vasoconstrictive agent released by platelets is serotonin. We attempted to aggregate the platelets directly on the brain surface and monitor the vascular response but we were then unable to visualize the vessels. Because of the method of preparation of the supernatant, it is possible that other labile vasoactive substances released by platelets may have vascular effects under conditions of in vivo platelet aggregation. The most prominent of such labile substances is thromboxane. Thromboxane is known to strongly contract strips from large cerebral arteries in vitro. Its effects on cerebral vessels in vivo have not been studied extensively. We attempted to study the effects of freshly prepared thromboxane from endoperoxide H2, and microsomal thromboxane synthetase. There was no effect or vasodilation. We concluded therefore that either thromboxane did not have any effect on cerebral vessels in vivo, or it was destroyed before reaching the vessels, or that its effects were counteracted by the vasodilatation caused by unaltered endoperoxide. More work is necessary to identify the action of thromboxane on cerebral vessels in vivo.

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