Contractile Responses of Pial Arteriols in Gerbils with Unilateral Carotid Ligation

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SUMMARY Studies were made in an effort to detect enhanced vasoconstriction ipsilateral to a ligated common carotid artery. No effect of ligation was found on the response to topically administered serotonin, norepinephrine or prostaglandin F/3, in the gerbil in spite of varying a number of parameters, including anesthesia, percent of inspired oxygen, time elapsed following ligation, or body temperature at time of examination. The response was not influenced by the presence or severity of stroke symptoms. A stronger and less specific constrictor, BaCl2, did elicit a difference between the contractile response of ligated and sham operated gerbils. In 13 of the 18 studies, BaCl2 produced a greater constriction in the ligated gerbils, and in 9 of these studies that difference was statistically significant. In only one study was there a statistically significant contrary result. The enhanced contractile response to BaCl2 could not be related to the presence or severity of symptoms or to any other of the variables mentioned above. This suggests that it was probably related to reduced intraluminal pressure distal to the ligation.

Methods

Mature gerbils of both sexes were used in approximately equal numbers as we found no influence of sex on the incidence of stroke. The right carotid was ligated (n = 300) or sham operation performed (n = 300) under ether or ketamine anesthesia. Two to 5 h after ligation a trachiotomy and craniotomy were performed under urethane anesthesia and the arachnoid surface exposed by stripping the dura. Pial arterioles were monitored with a Leitz Ultropak microscope and their diameters measured with an image splitter and TV monitor. The mean internal diameter of the arterioles we studied, measured after ligation or sham operation but before the local application of a contractile drug, fell between 30 and 55 μ. There was no difference between the initial mean arteriolar diameter of ligated and sham gerbils in most of the studies, the findings in an earlier preliminary investigation not being representative in this regard. There was no relationship between initial diameter and size of response.

After about 10 minutes drugs were applied to the pial surface in 1 ml volumes as previously described. The point of maximal narrowing was noted and the amount of constriction was expressed as a percent of baseline diameter

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\text{Percent constriction} = \left( \frac{\text{original diameter} - \text{new diameter}}{\text{original diameter}} \right) \times 100.
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Ten min after application of a single dose of either NOR, 5HT, or PGF2α when the monitored vessels had recovered from the effects of the drug, BaCl2 (5%) was applied in the same way as the drugs. Originally this was done to insure that vessels which failed to respond to the preceding drug were viable. All constricted to BaCl2. In 2 studies, only the BaCl2 was applied, after a stabilization period of 20 min.

Results

In all, 18 studies were performed. Each used no less than 10 ligated and 10 sham operated gerbils. In each study between 30 and 50% of the ligated gerbils had some symptoms ranging from ipsilateral ptosis to circling, gait disturbance and/or paralysis. Morphologic studies of other gerbils in our laboratory revealed ipsilateral hemispheric infarction in all gerbils displaying either circling, gait disturbance and/or paralysis. In 8 of the studies reported here only gerbils that showed symptoms more intense than ptosis were used. In 5 of the remaining studies, shams were compared with 2 groups of ligated gerbils, one group with the severe symptoms, and one with ptosis only (at least 10 gerbils in each group). There was no relationship between the degree of symptoms and the results.

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In 5 studies with NOR in doses of 0.1, 1, 10 or 100 μg/ml there was no effect of ligation on the size of the contractile response to NOR. In addition, we could not confirm the result of a single preliminary study showing an increased number of responding gerbils in the ligated group when compared with sham operated animals. Similar negative data were found in 9 studies with 5HT (0.001–10 μg/ml) and in 2 studies with PGF_{2α} (10–100 μg/ml). These negative results were generally obtained after ligation which had been performed under ketamine anesthesia, but in 4 studies ether was used rather than ketamine. In 13 studies observations were performed 4 h after ligation, in the remainder, 1, 2, 3, or 5 h after ligation, and, in one study, 3 days after ligation. In all but 2 studies the gerbil’s body temperature was maintained at 37°. In ten studies arterial blood was maintained hyperoxic by the addition of O₂ to the inspired air. No matter what the combination of anesthesia, time after ligation, arterial oxygenation or body temperature, ligation did not produce enhanced constriction to NOR, 5HT or PGF_{2α}. This was true even when observations of the arterioles revealed slow flow as in those studies where we selected for craniotomy only ligated gerbils with symptoms greater than ptosis (2 studies with NOR, 4 with 5HT and 2 with F_{2α}).

While we failed to find an effect of ligation on response to NOR, 5HT or F_{2α}, we noted increased magnitude of response to BaCl₂. In one study BaCl₂ produced a constriction of 78 ± 15% (M ± SD) in ligated gerbils as opposed to 54 ± 17% in sham operated gerbils (p < 0.01 t-test, 2-tailed). In 13 of the 18 studies, including one where BaCl₂ was applied without prior application of an amine or PGF_{2α}, constriction was observed in the ligated group (p = 0.048, sign test, 1 tail, where chance alone leads to the expectation that only 9 out of 18 studies would have such a result.) In 10 studies where the difference between ligated and sham groups was statistically significant (p < 0.05, 2 tailed t-tests) 9 of these 10 studies showed enhanced constriction in the ligated group. Further analysis failed to reveal a relationship between the response to BaCl₂ and any other defined parameter, such as application of a drug prior to BaCl₂, nature of preceding drug, nature of anesthesia during ligation, presence or absence of hyperoxegenation, presence of or severity of symptoms, or time following ligation.

**Discussion**

The results support the initial impression: we could not demonstrate an effect of ligation on the response to physiologic contractile stimuli such as NOR, 5HT or prostaglandin F_{2α}[PGF_{2α}], but in many of the same studies the response to BaCl₂ was enhanced. In view of the interest in the possible effects of vasoactive substances released during an ischemic stroke we believe our negative findings are of interest. We have no explanation for our failure to observe enhanced responses to 5HT while Bell et al. were able to observe such enhancement. We can, of course, point to differences between our animal model and theirs. Since we also failed to demonstrate an effect of ligation on the contractile response to NOR or F_{2α}, we cannot comment on the possibility that release of endogenous vasoactive material may occur during ischemia and that the presence of raised local concentrations of such material may enhance the constriction produced by application of additional amounts of the same material.

The enhanced response to BaCl₂ needs comment as it is obvious that we have not identified all the variables associated with such enhancement since it was not observed in 5 out of 18 studies. It is clear that such enhancement was a common occurrence, observed well beyond the dictates of chance. It is unclear why such a finding may occur with great statistical significance (e.g., p < 0.001, analysis of variance in one study of 75 ligated vs 75 sham animals) while there was no effect of ligation on the response to a natural constrictor (in this case NOR). It is possible that the magnitude of the response to the constriction is critical. Thus in our studies the constriction to NOR, 5HT or F_{2α} never averaged more than 34% of the resting diameter in sham operated gerbils while the constriction produced by BaCl₂ in sham gerbils never averaged less than 39% of resting diameter in any study, and in some studies reached 80% of resting diameter. It may be that the effects of ligation on the contractile response could only be demonstrated with intense contractile stimuli. As to the reason for the effect of ligation on the response to BaCl₂, it may be, as we originally suggested that this enhanced constriction reflects a reduced intraluminal pressure distal to the point of ligation. In fact, we have demonstrated that conditions leading to acute reductions in systemic blood pressure will be accompanied by increased contractile responses to BaCl₂. It is probable that all ligated animals, including those without symptoms, had reduced pressures in the peripheral distribution of the ligated carotid. The enhanced responses to BaCl₂ could not be related to the presence or severity of symptoms. In fact, in those ligated animals displaying only ptosis or no symptoms at all, prior unpublished morphologic data from this laboratory indicates that hemispheric infarction would not be identified in many of these animals, yet their inclusion in many of the studies failed to eliminate the enhanced contractile response to BaCl₂ found in the ligated groups. It is clear that we cannot ascribe this enhanced contractile response to a "stroke" or its consequences. These data should, therefore, suggest that enhanced contractile responses, observed in a stroke situation, are, in fact, not specifically related to tissue damage or to changes in tissue concentrations of contractile agents released.

**References**


Effects of Hypercapnia on Enhancement of Decreased Perfusion Flow
In Non-Infarcted Brain Tissues

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SUMMARY The effects of hypercapnia on enhancement of reduced cerebral perfusion were re-evaluated in areas of ischemia produced by occlusion of the canine middle cerebral artery. Perfusion was measured by $^{85}$Kr ($\beta$-ray) and $^{133}$Xe ($\gamma$-ray) clearances, fluorescein angiography and diameter measurement of arteries. Between 45 and 55 mm Hg of Paco$_2$ rCBF measured with both isotopes increased significantly. When Paco$_2$ was elevated above 55 mm Hg, there was a remarkable dissociation in the rCBF measured by both isotopes. Cortical blood flow measured by $^{85}$Kr clearance decreased and, conversely, rCBF measured by $^{133}$Xe continued to increase. Arteries of less than 50 $\mu$m in diameter in areas of ischemia dilated significantly during hypercapnia. At Paco$_2$ above 65 mm Hg, progressive sub-pial hemorrhage and extravasation of dye were observed as side effects of hypercapnia. The use of mannitol combined with hypercapnia appeared to be harmful. A Paco$_2$ level between 45 and 55 mm Hg increases perfusion in areas of mildly reduced rCBF.

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IN THE ACUTE STAGE of cerebrovascular occlusive disease, it is essential to prevent irreversible neuronal changes caused by ischemia by restoring decreased cerebral blood flow. Carbon dioxide, a potent cerebral vasodilator which does not reduce systemic blood pressure in the intact brain, has been considered a non-surgical method for enhancement of perfusion. In spite of numerous investigations in the last decade, the effect of CO$_2$ on improving CBF in ischemic brain still remains controversial. Inconsistency of the results is probably due to different methods or isotopes used to measure CBF and also due to differences in the degree of ischemia in animals or patients.

The effect of hypercapnia on restoration of reduced CBF was re-evaluated with beta-emitting $^{85}$Kr and gamma emitting $^{133}$Xe clearance techniques. In addition, the combined use of mannitol injection and CO$_2$ inhalation was tested in the same model.

Material and Methods

Procedure

Twenty-nine mongrel dogs, unselected as to age and sex, and weighing 19-24 kg were initially anesthetized with intravenous pentobarbital, 25 mg/kg. Additional pentobarbital, 25-50 mg, was given through a femoral vein cannula as necessary. The femoral artery was cannulated for continuous recording of blood pressure and sampling for blood gas analysis (I.L. Model 113 gas analyzer). After tracheal intubation, respiratory rate and volume were controlled and Flaxedil was given as needed. A small polyethylene catheter was introduced into the left lingual artery so that the tip of the catheter was at the junction with the external carotid artery. With the head of the dog fixed, a large
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