In Vivo and In Vitro Studies on the Cerebrovascular Dilatation Induced by Diazoxide in Normotensive And Renal Hypertensive Goats

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SUMMARY We studied the in vivo and in vitro effects of diazoxide on the cerebral circulation of 8 normotensive (mean arterial pressure = 100 mm Hg) and 5 renal hypertensive (mean arterial pressure = 146 mm Hg) goats. Injections of diazoxide (0.3-27 mg) into the internal maxillary artery of unanesthetized goats produced dose-dependent increases in cerebral blood flow (electromagnetic flowmeter), this effect being significantly higher in hypertensive goats. Intravenous injection of 5 mg/kg of diazoxide into normotensive goats increased cerebral blood flow 40 ml/min/100 g and mean arterial pressure dropped 22 mm Hg whereas in hypertensive goats cerebral blood flow was unchanged and mean arterial pressure decreased 50 mm Hg. The increase in heart rate due to intravenous diazoxide was similar in normotensive and hypertensive goats (35 beats/min). Cumulative applications of diazoxide (10\(^{-6}\) to 10\(^{-4}\)M) on isolated middle cerebral arteries produced dilatory responses both under resting conditions and after previous tonic contraction by serotonin. This relaxation was significantly greater in arterial segments from hypertensive goats. The results indicate that diazoxide exerts powerful dilatatory effects on cerebral vessels, both in vivo and in vitro, and that these effects are particularly evident in hypertensive animals.

EMERGENCY TREATMENT in a hypertensive crisis still raises many questions regarding the consequences on cerebral circulation. Hypotensive drugs devoid of effect on cerebral vessels risk decreasing cerebral blood flow and inducing cerebral ischemia, since hypertensive patients’ tolerance to blood pressure reductions is impaired. On the other hand, hypotensive drugs inducing cerebral vasodilatation may be harmful by increasing intracranial pressure or in producing arteriolar dilatation in elderly patients with arteriosclerotic stenosis, so that perfusion pressure may be reduced. It is important to know the direct effect of antihypertensive drugs on cerebral circulation in order to select proper treatment.

Diazoxide is a benzothiadiazine derivative devoid of diuretic action that is widely used in the treatment of hypertensive crisis. Data obtained from animal and human studies indicate that the mechanism of hypotensive action is direct relaxation of vascular smooth muscle. With regard to the cerebral vessels there are clinical observations indicating that cerebral blood flow is maintained within normal levels during the hypotensive action of diazoxide. Previous results from this laboratory indicated that diazoxide has a direct dilatory effect on cerebral vessels when it is administered through the internal maxillary artery of the unanesthetized goat. When the drug is injected intravenously, as a bolus, in doses of 5-7 mg/kg it produces hypotension while cerebral blood flow is increased, thus indicating a drop in cerebrovascular resistance.

This paper describes an investigation into the effects of diazoxide on the cerebral circulation of normotensive and renal hypertensive goats. The experiments in vivo were carried out in the unanesthetized animal, using an experimental preparation that permits the effects on cerebral blood flow of various interventions to be assessed in normal states. In addition, the
relaxing effects of diazoxide were evaluated on isolated middle cerebral arteries from both groups of animals.

Methods

Production of Hypertension

Five female goats, 35-45 kg, were anesthetized with intravenous 2% sodium thiopental and under sterile conditions a silk ligature with a triple loop, lumen width 2.5-3 mm, was applied to the left renal artery. The goats became hypertensive within one week and were left for a period of at least 2 months before further study. All 5 goats showed sustained mean arterial pressure of at least 130 mm Hg.

Cerebral Blood Flow Measurements

Cerebral blood flow was measured in 5 hypertensive goats 2-3 months after renal artery constriction and in 8 normotensive goats used as controls. In this species each internal maxillary artery, a branch of the external carotid artery, provides the total blood flow to each cerebral hemisphere via the rete mirabile; the vertebral arteries do not contribute to brain blood flow and the extracranial internal carotid artery is absent. Analysis of the distribution of radioactively-labeled microspheres in the cerebral circulation of the goat after the surgical procedure described by Reimann et al. indicates that nearly all of the blood carried by the internal maxillary artery passes directly to cerebral tissue. Extracerebral blood flow is minimal, less than 5% of total flow.

The operative procedure to measure cerebral blood flow has been described in detail before. Briefly, under 2% sodium thiopental anesthesia, the extracerebral vessels of one of the internal maxillary arteries were ligated and thrombosed with 1000 NIH units of thrombin (Thrombin, topical, Parke Davis and Co.) dissolved in 0.5 ml of saline. This maneuver produces an almost immediate obliteration of the ethmoidal, ophthalmic and buccinator arteries, thus eliminating blood flow to the eye and other facial tissues. This is confirmed upon recovery from surgery by the production of ipsilateral blindness. An electromagnetic flow transducer (Biotronex) was placed on the internal maxillary artery to measure blood flow to the ipsilateral cerebral hemisphere. A polyethylene catheter was inserted in the temporal artery and permanently fixed to permit injections of drugs directly into the internal maxillary artery; the same catheter was used to measure arterial blood pressure with a Statham transducer and occluder and the temporal artery catheter were led out subcutaneously and secured to the goat's horn. The experiments on the anesthetized goat started 2-3 days after the operative procedure, at which time the goats had fully recovered and were in a steady cardiorespiratory state.

The effects of diazoxide (Hyperstat, Schering) on cerebral blood flow, arterial blood pressure, and heart rate in normotensive control and hypertensive goats were evaluated by means of injections into a peripheral vein (5 mg/kg) or directly into the internal maxillary artery (0.3-27 mg) through the temporal catheter. Rapid injections were made in all cases, usually within 10-20 seconds. Intravenous injections were carried out in 5 hypertensive goats and in 4 normotensive goats and intra-arterial injections were performed in all goats from both groups. Cerebrovascular resistance was calculated as the mean arterial pressure in mm Hg divided by flow in ml/min.

In vitro Experiments

Four renal hypertensive goats and 4 normotensive goats were used in these experiments. The goats were killed by injecting intravenously 10 ml of saturated solution of potassium chloride. The brain was removed and the middle cerebral artery was carefully dissected out and cut into cylindrical segments 4 mm in length. The cylinder was set up in an organ bath containing 3 ml of Krebs-Henseleit solution continuously bubbled with 95% oxygen and 5% carbon dioxide to give a pH of 7.3-7.4, and was kept at 37°C. Two stainless steel pins, 150 μm diameter were introduced through the lumen of the arterial segment. One pin was fixed to the organ bath wall while the other was connected to a strain gauge for isometric recording. The latter pin was parallel to the former and movable, thus permitting the application of resting tension in a plane perpendicular to the long axis of the vascular cylinder. The recording system included a Universal transducing cell (UC1), a Statham microscale accessory (U15), and a Beckman type RS recorder. The composition of the Krebs-Henseleit solution was (mM): NaCl, 115; KCl, 4.6; CaCl₂, 2.5; KH₂PO₄, 1.2; MgSO₄·7H₂O, 1.2; NaHCO₃, 25; glucose, 11.1. A resting tension of 1 g was applied to the tissue and the segments were allowed to equilibrate during a period of 60 to 90 minutes. Diazoxide freshly diluted in distilled water was added to the organ bath in a volume up to 0.1 ml and cumulative dose-response curves to the drug were obtained. The effects of diazoxide were also studied under conditions of an active tonic contraction induced by previous addition of serotonin to the organ bath. Relaxation of the actively contracted arterial segment back to baseline tension represented 100% relaxation. The statistical analysis was done by means of the Student's t-test; a probability value of less than 5% was considered significant (p < 0.05).

Results

In Vivo Experiments

The table shows resting values for mean arterial blood pressure, cerebral blood flow, cerebrovascular
resistance, and heart rate in hypertensive goats and in control normotensive goats. Values for both arterial blood pressure and cerebrovascular resistance were about 45% higher in the hypertensive group than in the normotensive controls. There was no significant difference between the 2 groups of animals in cerebral blood flow and in heart rate.

Analysis of the results indicated that maximal effects of intravenous diazoxide occurred within the first 30 minutes post injection. The results of all the experiments are taken in this period of time. Figure 1 is a recording showing the effects of intravenous injection of 5 mg/kg of diazoxide on cerebral blood flow, arterial blood pressure, and heart rate in one normotensive and one hypertensive goat and figure 2 summarizes the data obtained from all the experiments.

**Figure 1.** Effects of intravenously administered diazoxide (5 mg/kg) on mean cerebral blood flow (Qc), arterial blood pressure (BP), and heart rate (HR), in one normotensive and one hypertensive goat. Deflections in Qc recording correspond to occlusion of the external carotid artery to obtain zero flow.

**Figure 2.** Effects of intravenous injection of diazoxide (5 mg/kg) on mean arterial blood pressure (MABP), cerebral blood flow (CBF), cerebrovascular resistance (CVR) and heart rate (HR) in 4 normotensive and 5 hypertensive animals. Values are means ± SEM.
Cerebral blood flow was increased in normotensive control goats, whereas in hypertensive goats it was maintained within normal values. In hypertensive goats arterial blood pressure dropped 50 mm Hg and in normotensive goats 22 mm Hg, on the average. Changes in heart rate and cerebrovascular resistance were similar in both groups of animals. All these values gradually returned to control 10 to 30 hours after the injection.

In some experiments in hypertensive goats a second intravenous injection of diazoxide was given shortly after the hypotensive effects of the first injection had become manifest. This was carried out in an effort to know whether or not cerebral blood flow could be maintained when blood pressure was reduced further. One of such experiments is illustrated in figure 3. A first injection of 5 mg/kg of diazoxide lowered blood pressure to normotensive levels, cerebral blood flow remained unchanged and the calculated cerebrovascular resistance decreased from 2.0 to 1.4 mm Hg/ml/min. A second injection of the same dose of diazoxide produced a more pronounced decrease in cerebral blood flow than in blood pressure and the cerebrovascular resistance increased. In another hypertensive goat in which a single injection of 5 mg/kg of diazoxide decreased arterial blood pressure below normotensive levels, cerebral blood flow was clearly diminished and became pressure dependent (fig. 4).

Injections of diazoxide into the internal maxillary
artery produced dose-dependent increases in cerebral blood flow, this effect being significantly greater in hypertensive goats. No detectable systemic effects were observed with the 4 lowest dosages used; however, a transient fall in arterial blood pressure and tachycardia were noted after the injection of the highest dose (27 mg). These systemic effects occurred after the increase in cerebral blood flow had become manifest. Figure 5 is an example of the cerebrovascular effects of intra-arterial administration of diazoxide in one normotensive and one hypertensive goat and figure 6 summarizes the data from all the experiments.

**In Vitro Experiments**

Under resting conditions cumulative applications of diazoxide produced a dilatory response which was dose-dependent. The left panel of figure 7 shows the effects of increasing concentrations of diazoxide on one arterial segment from one normotensive and from one hypertensive goat and the right panel summarizes the results obtained in 16 and 19 arterial segments from both groups of animals.

The relaxing effects of diazoxide were also evaluated under conditions of active tonic contraction induced by serotonin. Since differences in contraction can influence the degree of relaxation, arteries were contracted to equivalent tension before the addition of diazoxide. Concentrations of $10^{-4}$M and $3 \times 10^{-5}$M of serotonin were needed to achieve a contraction of 714 ± 50 mg in arterial segments from normotensive and hypertensive goats, respectively. Figure 8 shows an example of the relaxing effects of diazoxide on actively contracted arterial segments (left panel) and the concentration response curves obtained from 11 and 21 arterial segments obtained from normotensive and hypertensive goats, respectively (right panel).

As illustrated in figures 7 and 8, diazoxide-induced dilatation was significantly greater in arterial segments from hypertensive goats, both under resting conditions and after previous tonic contraction induced by serotonin.

**Discussion**

The advantages in the design of the present experiments both in vivo and in vitro are several. The study...
was conducted in unanesthetized goats to avoid the effects of anesthesia and acute surgical trauma. The use of a whole animal preparation has helped to ascertain continuous changes in cerebral blood flow and other hemodynamics at a time when the effects of diazoxide are manifest, and by injecting small amounts of the drug into the internal maxillary artery the direct cerebrovascular effects can be separated from those resulting from changes in overall systemic vascular resistance. The experiments on isolated middle cerebral arteries provided a useful way to determine the relaxing ability of diazoxide in vessels deprived of the tension present in vivo, which, by itself, may alter the degree of relaxation, and under conditions in which nervous and humoral influences are obviated.

The present results in unanesthetized goats indicate that direct administration of diazoxide into the internal maxillary artery produces cerebral vasodilatation without obvious accompanying effects on systemic arterial blood pressure or heart rate. This indicates that the augmentation of cerebral blood flow is due to a direct action of diazoxide on the cerebral vessels rather than a consequence of changes in systemic variables. This interpretation is consonant with the relaxation observed in isolated cerebral arteries in the presence of concentrations of diazoxide higher than $10^{-4} M$, and confirm similar results obtained in other vascular preparations.17,18 The present data also show that the dilatatory effect of diazoxide is more pronounced in hypertensive than in normotensive goats. Both the increase in cerebral blood flow after injections of diazoxide into the internal maxillary artery and the relaxation of the middle cerebral artery of hypertensive goats were significantly greater than those of the normotensive controls. Evidence in support of this contention is derived partially from the marked hypotensive effects of diazoxide in renal hypertensive dogs and DOCA hypertensive rats.4 Similarly, the effect of diazoxide in inhibiting Ba$^{++}$ induced contraction is greater in isolated aortic segments from DOCA hypertensive rats than in aortic segments from control rats.19

Intravenous injection of diazoxide in clinical doses (5 mg/kg) produced changes in blood pressure and heart rate similar to those previously reported in
anesthetized animals and in man.20-23 With respect to the cerebrovascular effects of intravenous diazoxide, the present experiments show that values for cerebral blood flow are increased in the normotensive goats whereas they are maintained in the hypertensive goats. This difference in the direction of cerebral blood flow between the 2 groups of animals is probably due to the size of the arterial pressure drop observed in hypertensive goats (50 mm Hg) after intravenous administration of diazoxide as compared to that obtained in normotensive goats (20 mm Hg). It is interesting to point out that a second injection of diazoxide in hypertensive goats produces, in most, a further drop in blood pressure; however, the second injection is accompanied by a decrease in cerebral blood flow indicating that a maximal or near maximal dilatation is taking place. Under such circumstances cerebral blood flow can become arterial pressure-dependent and, hence, as the pressure decreases so does the cerebral blood flow. This situation may also be observed with only one injection of diazoxide (fig 4) due to the greater hypotensive effect of the drug in these animals. Therefore, if diazoxide has any salutary effects on blood supply to the brain in treatment of hypertension, care should be taken not to lower blood pressure beyond levels in which cerebral blood flow can become arterial pressure-dependent.

Closely related to the foregoing discussion is the finding that intravenous administration of diazoxide in hypertensive goats did not change cerebral blood flow significantly while injections into the internal maxillary artery in the same animals produced a marked augmentation of cerebral blood flow. The reason for this apparent discrepancy is probably the large decrease in cerebral perfusion pressure after intravenous administration. This is not observed when the drug is locally administered. The present experiments both in vitro and in vivo indicate that diazoxide exerts powerful dilatatory effects on cerebral vessels and that these effects are particularly evident in hypertensive animals. Intravenous administration of 5 mg/kg of diazoxide into normotensive goats increases cerebral blood flow whereas in hypertensive goats the same dose of diazoxide maintains cerebral blood flow in the face of a large drop in arterial blood pressure. If the cerebrovascular action of diazoxide is to be applied to hypertensive man one must bear in mind that larger doses of the drug may cause hypotension “in excess” and thus interfere with the proper blood supply to the brain.

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