Analysis of Cerebrovascular Action of Diazoxide in Conscious Goats

G. Dieguez, M.D., B. Gomez, M.D., and S. LLuch, M.D.

SUMMARY The effects of diazoxide on cerebral blood flow were evaluated in unanesthetized goats under control conditions and after selective blockade of adrenergic or cholinergic receptors in cerebral vessels. Injections of diazoxide (1–27 mg) into the internal maxillary artery produced dose-dependent increases in cerebral blood flow, an increase of 90% occurring with the highest dose. Administration of propranolol, procnanol, or atropine into the internal maxillary artery did not modify the cerebrovascular response to diazoxide. In reserpine-treated animals the cerebral effects of diazoxide were also unchanged. Intravenous injections of diazoxide (150–400 mg) produced sustained hypotension and tachycardia whereas cerebral blood flow was maintained within normal values or increased slightly. The normal cerebral vasocostriction obtained with injections of norepinephrine directly into the internal maxillary artery was unaffected during the diazoxide induced-hypotension. These findings show that diazoxide exerts a powerful vasodilatory effect on cerebral vessels through mechanisms other than blockade of alpha-adrenergic receptors or inhibition of adrenergic activity. The results also indicate that activation of beta-adrenergic or atropine-sensitive vascular receptors in the cerebral response to diazoxide is negligible.

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CLINICAL OBSERVATIONS show the efficacy and safety of the benzothiadiazine derivative, diazoxide, in the treatment of severe hypertension from any cause.1-7 Data obtained from animal and human studies indicate that the mechanism of its hypotensive action is direct relaxation of vascular smooth muscle.8-10 Together with an interaction, under certain experimental conditions, with adrenergic receptors.5, 6, 11, 12

There are clinical observations and actual blood flow measurements indicating that cerebral blood flow is maintained during the hypotensive action of diazoxide.13, 14 In one of these reports14 the possibility was raised whether the drug might have direct dilatatory effects on the cerebral vasculature, as has been demonstrated in other vascular regions.15, 16, 17 The purpose of this communication is to introduce data on the effects of diazoxide on the cerebral circulation of unanesthetized animals. In addition, the role played by adrenergic and cholinergic receptors present in cerebral vessels was studied by selective blockade of one brain hemisphere with specific antagonists. All the experiments were carried out in the goat, using an experimental preparation that permits the effects on cerebral blood flow of various interventions to be assessed in normal states.17-20

Methods

Eleven female goats weighing between 32 and 45 kg were used. 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.17, 21, 22 The circle of Willis in the goat is similar to that of man except that the blood flows in a caudal direction in the basilar artery, which has only insignificant communications with the vertebral artery.17, 21 Analysis of the distribution of radioactively labeled microspheres in the cerebral circulation of the goat after the surgical procedure described by Reimann et al.17 indicates that nearly all of the blood carried by the internal maxillary artery passes directly to cerebral tissue.20 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.17 Briefly, under 2% sodium thiopental anesthesia, the extracerebral vessels of one of the internal maxillary arteries were ligated and thrombosed with 1,000 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, opthalmic, and buccinator arteries, thus eliminating blood flow to the eye and other facial tissues. An electromagnetic flow transducer (Biotronex, Silver Spring, MD) 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 injection or infusion of drugs directly into the internal maxillary artery; the same catheter was used to measure arterial blood pressure with a Statham transducer. A snare-type occluder was placed on the external carotid artery to obtain zero flow baseline.

Heart rate was measured from the arterial pressure pulse with a rate meter. Cerebral blood flow, arterial blood pressure, and heart rate were recorded on a Beckman recorder. The experiments on the unanesthetized animal started 2–3 days after the operative procedure, at which time the goat had fully recovered and was in a steady cardiorespiratory state.

Diazoxide (Hyperstat, Schering) was injected into the internal maxillary artery (1 to 27 mg) in 5 goats and into a peripheral vein (5–10 mg/kg) in another 3 goats; in the remaining 3 goats both intra-arterial or intravenous administration was used. Rapid injections were made in all experiments, usually within
10-20 seconds. The effects of direct administration of diazoxide into the internal maxillary artery were evaluated before and after alternate adrenergic or cholinergic blockade of cerebral vessels. A period of at least 24 h was allowed between adrenergic (alpha or beta) and cholinergic inhibition. Alpha-adrenergic blockade of the cerebral circulation was produced in 5 goats by slow infusion of phentolamine (Regitin, Ciba) into the internal maxillary artery (1-1.5 mg in 1 ml of saline over 10 minutes); propranolol (1 mg, Sumial ICI Farma) was used in a similar manner in 5 goats; and atropine (1-2 mg, Atropina Miro) was used in 3 goats. Reserpine (Serpasil, Ciba) was infused intravenously in 3 doses of 1 mg each, 24 h apart, in 2 goats.

The dose of phentolamine used effectively blocks the cerebral vasoconstriction produced by tyramine or cervical sympathetic nerve stimulation whereas propranolol and atropine significantly decrease the vasodilatation which follows the administration of isoproterenol and acetylcholine, respectively. Similarly, the dose of reserpine used in these experiments reduces the catecholamine content of cerebral vessels to undetectable levels and inhibits the cerebral vasoconstriction which occurs with tyramine and cervical sympathetic nerve stimulation.

Arterial blood was analyzed before and during the effects of diazoxide for pH, PCO2, and PO2 by standard electrometric methods (Corning Scientific Instruments, model 165, Medfield, MA). The statistical analysis was done using Student's t-test; a probability value of less than 5% was considered significant.

Results

Injections of Diazoxide into the Internal Maxillary Artery. In all the experiments the administration of diazoxide (1-27 mg) directly into the internal maxillary artery produced dose-dependent increases in cerebral blood flow. No detectable systemic effects were observed with the 3 lowest dosages used; however, a transient fall in arterial blood pressure and tachycardia were noted after the injection of the highest dose. These systemic effects were noted after the increase in cerebral blood flow had occurred. Figure 1 is a representative example of the effects of increasing doses of diazoxide on cerebral blood flow, systemic arterial blood pressure, and heart rate in one goat.

Selective alpha-adrenergic blockade of one brain hemisphere with phentolamine (1-1.5 mg) produced an increase in cerebral blood flow of 26% on the average without altering systemic arterial blood pressure or heart rate. Injections of diazoxide under these conditions increased cerebral blood flow to a level which did not differ significantly (p > 0.05) from that found in the untreated state. Blockade of beta-adrenergic receptors with propranolol (1-2 mg) reduced cerebral blood flow by 12% but the cerebrovascular response to diazoxide was not significantly different (p > 0.05) from that obtained before treatment. Administration of atropine in doses of 1-2 mg caused no significant alteration (p > 0.05) of resting cerebral blood flow and did not appreciably affect the cerebral vasodilatation induced by diazoxide. Figure 2 shows actual recordings of the effects of diazoxide on cerebral blood flow before and after selective blockade of adrenergic (alpha or beta) or cholinergic vascular receptors. Table 1 summarizes the data. Treatment of 2 animals with reserpine (1 mg i.v./day for 3 days) produced a significant drop in both arterial blood pressure and heart rate but cerebral blood flow was maintained within normal values. The effects of intrarterial administration of diazoxide in these animals did not differ significantly from those obtained before reserpine treatment (fig. 3).

Intravenous Injections. Intravenous injections of diazoxide (5-10 mg/kg) resulted in consistent significant decreases in systemic blood pressure. As illustrated in figure 4 this effect was accompanied by slight increases in cerebral blood flow and a marked rise in heart rate. These changes occurred immediately after the injection and gradually returned to control levels within

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**Figure 1.** Sections of an experimental record showing the effects of increasing doses of diazoxide injected into the internal maxillary artery on cerebral blood flow (CBF), arterial blood pressure (AP), and heart rate (HR) in one unanesthetized goat. Deflections of CBF and AP records correspond to occlusion of external carotid artery to obtain zero flow.
TABLE 1  Effects of Diazoxide on Cerebral Blood Flow under Different Experimental Conditions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CBF (ml/min per 100 g)</th>
<th>Diazoxide mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (n = 8)</td>
<td>122 ± 6.6</td>
<td>133 ± 7.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>161 ± 9.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>196 ± 10.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>233 ± 12.6</td>
</tr>
<tr>
<td>Phentolamine (n = 5)</td>
<td>154 ± 9.6</td>
<td>173 ± 12.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>186 ± 13.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>203 ± 13.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>260 ± 18.4</td>
</tr>
<tr>
<td>Propranolol (n = 5)</td>
<td>100 ± 8.5</td>
<td>126 ± 8.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>148 ± 10.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>184 ± 9.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>217 ± 10.0</td>
</tr>
<tr>
<td>Atropine (n = 4)</td>
<td>126 ± 6.3</td>
<td>139 ± 7.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>154 ± 8.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>190 ± 14.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>217 ± 15.2</td>
</tr>
</tbody>
</table>

n = number of goats. Values are means ± SEM.

TABLE 2. Cerebral Blood Flow (CBF), Mean Arterial Blood Pressure (MAP), and Heart Rate (HR) Before and 6 Minutes After i.v. Administration of 7 mg/kg Diazoxide

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diazoxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBF (ml/min per 100 g)</td>
<td>121 ± 4.6</td>
<td>141 ± 10.2*</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>97 ± 3.9</td>
<td>73 ± 2.8*</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>73 ± 6.2</td>
<td>125 ± 14.6*</td>
</tr>
</tbody>
</table>

Values are means ± SEM obtained from 6 goats. *Statistically different from control (p < 0.05).

effects of intravenous diazoxide in 4 goats. Figure 5 shows that diazoxide failed to alter the normal cerebral vasoconstrictor response to norepinephrine.

Discussion

The experimental preparation used is advantageous because the direct effect of drugs on cerebral blood flow can be assessed in the conscious animal by injection into the internal maxillary artery eliminating changes in blood pressure and heart rate.

The data indicate that diazoxide is a potent cerebral vasodilator acting directly on cerebral vessels. Relatively small doses of diazoxide administered into the arterial supply to the brain produce sizeable increases in cerebral blood flow without obvious accompanying changes in arterial blood pressure or heart rate. When larger doses were used the increase in cerebral blood flow occurred 10 to 20 seconds before the fall in blood pressure. This sequence of events indicates that the augmentation of cerebral blood flow induced by diazoxide is due to a direct action on the cerebral vessels rather than as a consequence of changes in systemic variables. This interpretation is consonant with findings in animal studies and in man.4, 10, 15, 16, 28, 29 The degree of cerebral vasodilatation...
tion attained with diazoxide is even higher than that obtained with sodium nitroprusside\textsuperscript{28} and comparable to that resulting from superior cervical ganglionectomy\textsuperscript{29} or CO\textsubscript{2} inhalation,\textsuperscript{30} under the same experimental conditions.

Selective alpha blockade of cerebral vessels increased resting cerebral blood flow as a consequence of loss of part of alpha-adrenergic tone normally existing in cerebral vessels.\textsuperscript{15, 28} Under these circumstances the effects of diazoxide were unchanged, thus indicating that inhibition of adrenergic vasoconstrictor tone does not participate in the vasodilatation induced by the drug. The normal cerebrovascular response to diazoxide after reserpine treatment, which reduces the catecholamine content of cerebral vessels to undetectable levels,\textsuperscript{31, 32} further supports the concept that adrenergic activity is not an essential requisite for the dilatatory effects of diazoxide. Intra-arterial administration of propranolol or atropine in doses which block the cerebral dilatation produced by isoproterenol\textsuperscript{18} or acetylcholine\textsuperscript{33} did not affect the normal vascular response to diazoxide. These results indicate that the activation of beta-adrenergic or atropine-sensitive receptors is of minor importance in the cerebrovascular response to diazoxide. Evidence in support of these results is derived partly from data which show that blockade of adrenergic or cholinergic receptors does not affect the development of diazoxide-induced hypotension.\textsuperscript{3, 10, 15, 28} Despite results reported in dog gracilis muscle\textsuperscript{16} and in the renal hypertensive rat,\textsuperscript{19} our data show that diazoxide did not attenuate the cerebral vasoconstrictor response to norepinephrine. This finding, together with the observation that phentolamine or reserpine do not change the cerebral effects of diazoxide, indicates that diazoxide does not act, to a great extent, by blockade of alpha-adrenergic receptors or inhibition of adrenergic activity. Similar conclusions have been reached from other animal experiments\textsuperscript{15} and observations in man.\textsuperscript{6}

Intravenous injections of diazoxide in the goat in clinical dosages (5 to 10 mg/kg) produce changes in blood pressure and heart rate similar to those previously reported in anesthetized animals\textsuperscript{10, 15} and in man.\textsuperscript{1, 4-6} The cerebrovascular effects of diazoxide, in these experiments, show that resting values for cerebral blood flow are maintained in the face of a lowered arterial blood pressure, thus indicating that a drop in cerebrovascular resistance occurs. Our data also show that hypotension induced by diazoxide produces cerebrovascular effects different from those appearing with hemorrhagic or ganglionic blockade hypotension in unanesthetized goats. During hemorrhage cerebral blood flow parallels the fall in systemic arterial blood pressure\textsuperscript{34} and after ganglionic blockade with trimetaphan (Arfonad) cerebral blood flow is maintained, probably due to the progressive loss of an existing sympathetic tone in the cerebral blood vessels.\textsuperscript{35} However, after intravenous injection of diazoxide the maintenance of cerebral blood flow is probably the result of a direct effect of the drug on the cerebral vessels. The mechanisms causing cerebral vasodilatation are probably similar to those described in other vessels and involve a direct relaxing effect on vascular smooth muscle.\textsuperscript{10, 16, 35}

Acknowledgment

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