Analysis of Cerebrovascular Action of Diazoxide in Conscious Goats

G. Dieguez, M.D., B. Gómez, M.D., and S. Lucho, 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 phenolamine, propranolol, 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 vasconstriction 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.

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. 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. 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 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, ophthalmic, 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 Miró) 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 (p < 0.05).

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 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 from that obtained before treatment. Administration of atropine in doses of 1-2 mg caused no significant alteration 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 intravenous 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 20 minutes.
TABLE 1. Effects of Diazoxide on Cerebral Blood Flow under Different Experimental Conditions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>1</th>
<th>3</th>
<th>9</th>
<th>27</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (n = 8)</td>
<td>122 ± 6.6</td>
<td>133 ± 7.2</td>
<td>161 ± 9.4</td>
<td>196 ± 10.3</td>
<td>233 ± 12.6</td>
</tr>
<tr>
<td>Phentolamine (n = 5)</td>
<td>154 ± 9.6</td>
<td>173 ± 12.2</td>
<td>186 ± 13.6</td>
<td>203 ± 13.0</td>
<td>260 ± 18.4</td>
</tr>
<tr>
<td>Propranolol (n = 5)</td>
<td>100 ± 8.5</td>
<td>126 ± 8.0</td>
<td>148 ± 10.4</td>
<td>184 ± 9.8</td>
<td>217 ± 10.0</td>
</tr>
<tr>
<td>Atropine (n = 4)</td>
<td>126 ± 6.3</td>
<td>139 ± 7.2</td>
<td>154 ± 8.3</td>
<td>190 ± 14.7</td>
<td>217 ± 15.2</td>
</tr>
</tbody>
</table>

* n is number of goats. Values are means ± SEM.

DIAZOXIDE (mg)

Goat 4 B

CBF 100 ml/min

Goat 6 B

CBF 100 ml/min

Goat 4 B

CBF 100 ml/min

FIGURE 2. Effects of intra-arterial injection of diazoxide on cerebral blood flow before and after administration of phentolamine, propranolol, or atropine into the internal maxillary artery.

10–30 h. Table 2 summarizes all values for cerebral blood flow, mean arterial blood pressure, and heart rate obtained from 6 goats before and after intravenous injection of diazoxide. Arterial blood samples obtained from 8 goats before diazoxide and during the hypotensive state did not show any significant difference in pH, PCO₂, and PO₂ values.

To investigate the possibility that diazoxide might decrease the normal cerebrovascular response to alpha-adrenergic stimulation, injections of norepinephrine were carried out directly into the internal maxillary artery before and during the hypotensive state.

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>

* Statistically different from control (p < 0.05).

DIAZOXIDE mg

Goat 4 B

CBF 100 ml/min

AP 150 mm Hg

HR 50 beats/min

RESERPINE

Goat 9B

CBF 100 ml/min

AP 150 mm Hg

HR 50 beats/min

FIGURE 3. Effects of diazoxide on cerebral blood flow, arterial blood pressure, and heart rate in one unanesthetized goat before and after treatment with reserpine. Symbols as in fig. 1.

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 injections 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, 12, 16, 26, 37 The degree of cerebral vasodilatation...
tion attained with diazoxide is even higher than that
tained with sodium nitroprusside and comparable
to that resulting from superior cervical ganglionec-
tomy or CO₂ inhalation, under the same experi-
mental conditions.

Selective alpha blockade of cerebral vessels in-
creased resting cerebral blood flow as a conse-
quency of loss of part of alpha-adrenergic tone normally exist-
ing in cerebral vessels. Under these circumstances 
the effects of diazoxide were unchanged, thus indi-
cating 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 undetect-
able levels, further supports the concept that 
adrenergic activity is not an essential requisite for the 
dilatatory effects of diazoxide. Intra-arterial adminis-
tration of propranolol or atropine in doses which 
block the cerebral dilatation produced by isopro-
tereno or acetylcholine 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 cere-
brovascular response to diazoxide. Evidence in sup-
port of these results is derived partly from data which 
show that blockade of adrenergic or cholinergic recep-
tors does not affect the development of diazoxide-
induced hypotension. Despite results reported 
in dog gracilis muscle and in the renal hypertensive rat, our data show that diazoxide did not attenuate the cerebral vasoconstrictor response to norepineph-
rine. 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 ex-
periments and observations in man.

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 and in 
man. The cerebrovascular effects of diazoxide in 
these experiments, show that resting values for cere-
bral 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 cere-
brovascular effects different from those appearing 
with hemorrhagic or ganglionic blockade hypoten-
sion in unanesthetized goats. During hemorrhage cere-
bral blood flow parallels the fall in systemic arterial 
blood pressure and after ganglionic blockade with 
trimetaphan (Arfonad) cerebral blood flow is main-
tained, probably due to the progressive loss of an ex-
ist sympathtetic tone in the cerebral blood vessels. 
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 
veins. The mechanisms causing cerebral vasodilata-
tion are probably similar to those described in other 
veins and involve a direct relaxing effect on vascular 
smooth muscle.

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