blood flow in cats. Pflügers Arch 333: 337-351, 1972

PROPRANOLOL'S POTENTIAL as a protective agent against tissue injury has been noted in experimental myocardial, renal and early acute focal cerebral ischemia. The purpose of the present investigation was to study further the effects of racemic (d,l) propranolol on blood-brain barrier permeability, morphological changes, cortical electrical activity, and regional cerebral blood flow (rCBF) in experimental focal cerebral ischemia. Thirty adult cats, anesthetized with nitrous oxide, underwent 6 hours of right middle cerebral artery (MCA) occlusion. Fifteen cats were untreated. Fifteen cats were given a continuous infusion of racemic propranolol (1 mg/kg/hr) for 7 hours beginning 1 hour before MCA occlusion and a 4 mg/kg bolus immediately before occlusion, both directly into the right carotid artery. Right Sylvian rCBF did not change continuously. A heating pad was placed over the trunk and mechanical ventilation instituted. The right femoral artery and vein were cannulated. Tubocurarine chloride (1 mg/kg) was given subcutaneously. Needle electrodes were placed subcutaneously for continuous EKG monitoring. Mean arterial blood pressure was monitored continuously. A heating pad was placed over the trunk to maintain core temperature at 37°C and was monitored continuously by rectal thermometer. Arterial

SUMMARY
Propranolol's potential as a protective agent against tissue injury has been noted in experimental myocardial, renal and early acute focal cerebral ischemia. The purpose of the present investigation was to study further the effects of racemic (d,l) propranolol on blood-brain barrier permeability, morphological changes, cortical electrical activity, and regional cerebral blood flow (rCBF) in experimental focal cerebral ischemia. Thirty adult cats, anesthetized with nitrous oxide, underwent 6 hours of right middle cerebral artery (MCA) occlusion. Fifteen cats were untreated. Fifteen cats were given a continuous infusion of racemic propranolol (1 mg/kg/hr) for 7 hours beginning 1 hour before MCA occlusion and a 4 mg/kg bolus immediately before occlusion, both directly into the right carotid artery. Right Sylvian rCBF did not change significantly different in the treated and untreated groups. Carbon filling defects and vital dye (i.e., Evans blue and fluorescein) extravasation were less severe in the propranolol treated animals. Light microscopic findings demonstrated no difference in infarct size between the two groups. The findings suggest that at doses given, racemic propranolol does not exert a protective effect upon cerebral tissue subjected to 6 hours of incomplete ischemia.

Treatment of Acute Focal Cerebral Ischemia With Propranolol
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blood was sampled prior to each blood flow measurement to maintain a PaCO$_2$ of 30–35 mmHg and PaO$_2$ above 100 mmHg.

Electroencephalography

Scalp and temporalis muscles were incised and retracted from the skull. Small holes (1.5 mm) were drilled bilaterally 1 cm from the midline at distances of 0.4 cm, 1.4 cm, and 2.4 cm posterior to the junction of the sagittal and coronal sutures. Stainless steel bolt electrodes (#2-56) were inserted to a depth contacting the dura. The position of these electrodes corresponded with the border zone between the MCA and anterior cerebral arteries. A midline reference electrode was inserted into the frontal air sinus. A ground electrode was implanted into the left temporalis muscle. Tracings were recorded on a Grass model 6 electroencephalograph with amplitudes 20% down at 1 and 70 Hz. The electroencephalogram (EEG) was recorded for 2 minute periods before, during, and after MCA occlusion. Tracings were recorded hourly thereafter and prior to each rCBF measurement.

Surgical Preparation

A small catheter was inserted into the right lingual artery and advanced to the junction of the right carotid artery for subsequent injection of Xenon-133 and for direct administration of the therapeutic agent. The heads were shaved and placed in a headholder which provided unobstructed access to the right orbit. The orbit was exenterated and a small craniectomy was performed along the superolateral margin of the optic foramen. Using microsurgical techniques, the dura and arachnoid membranes were opened. The proximal segment of the right MCA was dissected from the adjacent structures in preparation for application of a miniature aneurysm clip.

Cerebral Blood Flow

The rCBF was measured by the Xenon-133 clearance technique. The $^{133}$Xe window (81 keV) was determined with a multichannel analyzer. A collimated 1.5 cm sodium iodide crystal recessed 5.0 cm was applied to the skull overlying the right Sylvian cortex. $^{133}$Xe (200 µCi in 0.5 ml normal saline) was injected rapidly into the right lingual artery catheter and measurements were recorded on a multichannel analyzer for 10 minutes. Kinetic analysis was used to determine the rCBF. Measurements were performed immediately before and after occlusion and repeated 3 hours and 6 hours after occlusion.

MCA Occlusion

The right MCA was occluded with a miniature aneurysm clip for 6 hours.

Treatment Groups

Thirty cats were alternately assigned to the untreated and racemic propranolol groups. Immediately prior to MCA occlusion the 15 treated cats received a 1 ml bolus of racemic (d, l) propranolol (4 mg/kg) injected into the right carotid artery via the lingual artery catheter. Beginning 1 hour before MCA occlusion and continuing 6 hours after occlusion, racemic propranolol in saline suspension (1 mg/kg/hr) was administered through the lingual artery by an IMED 965 microinfusion pediatric pump (IMEP Corp., 9925 Carroll Canyon Rd., San Diego, CA 92131). Equivalent amounts of normal saline were administered to the 15 untreated cats.

Perfusion

Five and one-half hours after MCA occlusion, Evans blue dye and sodium fluorescein (i.e., 100 mg in 1 ml) were administered over several minutes through the femoral vein. At the end of the 6 hour ischemic period, the aneurysm clip was removed, and a thoracotomy was performed. The right atrium was incised, the descending aorta was clamped and 150 ml of saline infused into the ascending aorta at 120 mmHg pressure. Immediately thereafter, 150 ml of colloidal carbon and 150 ml of phosphate buffered 7.4 pH, 4% formaldehyde in distilled water at 120 mmHg was infused into the aorta. The brain of each cat was removed and placed in 4% formaldehyde, 7.4 pH, for at least 48 hours prior to pathological preparation.

Examination of Brains

The presence or absence of Evans blue and fluorescein staining was recorded. The distribution of carbon staining was graded according to Crowell and Olson. Paraffin embedded coronal hemispheric sections, stained with hematoxylin and eosin and periodic acid Schiff, were examined by light microscopy. In coronal sections of each hemisphere 3 mm posterior to the temporal lobe tip, the cross-sectional area of gray matter where moderate and severe neuronal alterations (Grade II, III) predominated was measured by a blinded observer using a Keuffel and Esser planimeter (Keuffel and Esser Company, New York, NY). Statistical analysis of the percentage of ischemic gray matter was performed using the unpaired t-test.

Results

Vital Signs

Systemic stability was maintained in the 30 cats undergoing right MCA occlusion. Arterial blood pressure was similar in the two groups while heart rate was somewhat reduced in animals treated with propranolol (table 1). Hematocrit was 35 ± 1% in the untreated group and 37 ± 6% in the treated group. Completely randomized design with split plot treatment disclosed no statistical difference in the two groups for blood pressure, heart rate or hematocrit.

Regional Cerebral Blood Flow

Results of $^{133}$Xe clearance are displayed in figure 1. A marked reduction in rCBF occurred immediately subsequent to right MCA occlusion in both treated and untreated groups. No significant difference in rCBF was noted at 3 hours and 6 hours after occlusion. The rCBF dropped to <14 ml/100 gm/min in 3 propranolol
treated animals immediately after clipping. In each of these animals rCBF improved and in 2 others the rCBF remained considerably below the mean. A similar drop of rCBF to 14 ml/100 gm/min was noted in 1 untreated animal immediately after clipping. Eight propranolol treated cats and 3 control cats were found to have rCBF <18 ml/100 gm/min on one or more determinations. Analysis of variants utilizing repeated measures design noted no significant differences.

EEG Findings

The EEG background consisted of 5–10 Hz activity of up to 50 μV. In addition, 15–30 Hz 15–20 μV activity and occasional bursts of 10–15 Hz 50–70 μV rhythms occurred in both untreated and treated animals after pre-occlusion bolus.

The amplitude of the 5–10 Hz activity on the occluded side was analyzed and expressed as a percentage of that on the non-occluded side in order to correct for bilateral EEG changes during the procedure.

Occluded/non-occluded voltage ratios, expressed as percentages at pre-occlusion, post-occlusion, 3 hours and 6 hours post-occlusion were 79.9 ± 11.3%, 64.6 ± 12.9%, 61.9 ± 14.9%, 82.7 ± 19.3% in the untreated group and 91.5 ± 24.7%, 70.7 ± 25.7%, 62.7 ± 18.3% and 59.9 ± 15.1% in the treated group. No significant difference was observed.

Morphological Studies

Macroscopic Findings

The right MCA and its branches were well filled with carbon fixative solution, confirming vessel reperfusion after aneurysm clip removal. Gross tissue swelling in MCA territory was present in 15/15 untreated and 14/15 propranolol treated cats. The mean right to left shift of midline structures was 1 ± .5 mm in both groups. Marked carbon perfusion defects including cortex and caudate nucleus were present in 9 untreated and 9 treated animals (table 2). Moderate filling defects were noted in 6 cats in the control group and 3 cats in the propranolol treated group. One propranolol treated animal showed impaired carbon filling restricted to the right caudate nucleus. Two propranolol treated animals displayed no cortical or subcortical filling defects. Fluorescein staining was present in all specimens. Evans blue extravasation was marked in 5, moderate in 7 and mild in 1 of the untreated controls; while marked in 2, moderate in 6 and mild in 1 of the propranolol treated brains (table 3). Two untreated and 6 treated animals showed no Evans blue staining. No statistical difference was noted using the Mann-Whitney U test ($p < .1$).

Microscopic Findings

Severe ischemic neuronal alterations were present in the caudate nucleus and/or cortex in all cats. Substantial astrocytic swelling, capillary narrowing and obstruction were invariably seen in areas where neuronal changes were noted. The percentage of ischemic gray matter cross-sectional area was 43.9 ± 25 in untreated animals and 57.9 ± 19 in treated animals. The difference in size of infarct between untreated and treated animals did not achieve statistical significance as determined by the unpaired t-test.

Discussion

Advances in radioligand binding have allowed identification of beta-adrenergic receptors in various tissues including erythrocytes, heart, and brain. Although distributed throughout the brain, the highest density of beta receptors is found in the cerebral cortex and corpus striatum. Findings corroborated by more recent autoradiographic preparations.
tion of beta-adrenergic receptors to neurons and/or glial cells has not been determined. Presence of a high density of beta receptors in regions with no adrenergic input, as well as finding that there is no beta-adrenergic receptor density change subsequent to kainic acid induced neuronal cell death, has prompted suggestion of predominant glial rather than neuronal localization in corpus striatum.

Both radioligand binding and beta receptor stimulated adenylate cyclase in purified preparations of cerebral blood vessels have verified the presence of vessel beta receptors. These receptors comprise only a fraction of cerebral beta receptors as microvessels constitute <1% of whole brain homogenate, and 1-2% of cortical gray matter, and as beta receptor density of microvessel fraction is much less than in whole brain homogenate. Culvenar and Jarrott have presented evidence that although the density of the beta receptors in cortical microvessels was half that in grey matter membranes, the affinity for binding antagonist was six times greater. Both beta1 and beta2 subtype specificity have been suggested for cerebral microvasculature.

The extent and importance of adrenergic innervation on cerebral vessels and cortical membranes has yet to be clarified. Extrinsic innervation of cerebral vessels via the superior cervical ganglion aids in preserving cerebral autoregulation at extremes of the autoregulatory curve. An intrinsic system originating in the pontine locus coeruleus may mediate vasoconstriction via alpha receptors. Dahlgren et al questioned this system's regulating function as electrothermic and 6-hydroxydopamine lesions to the nucleus locus coeruleus did not affect resting state cerebral blood flow (CBF) and oxygen consumption (CMRO2) or influence responses to hypercapnia and hypoxia. The intrinsic noradrenergic system has, however, been shown to affect neuronal discharges. Inhibition of firing of cerebellar, cortical, and hippocampal neurons results from electrical stimulation of locus coeruleus or from microiontophoretically applied norepinephrine. Sotolol, a beta antagonist, has been shown to block this inhibitory influence of the locus coeruleus. Fuxe et al found that l-propranolol reduced noradrenergic turnover in rat brain, an effect that could not be related to noradrenergic uptake and release or noradrenergic receptor sites. They postulated that l-propranolol may reduce noradrenergic turnover by acting as an agonist on noradrenergic neurons, including cerulocortical systems.

The influence of circulating catecholamines with this neuroanatomical substrate has recently been investigated. Olendorf found little blood-brain barrier permeability for catecholamines. Others have demonstrated that disruption of blood-brain barrier by catecholamine infusion and rapid increases in blood pressure or release of endogenous norepinephrine may be associated with marked increase in CBF and CMRO2. Beta-adrenergic effect on cerebral metabolism and blood flow has been suggested since racemic propranolol has been shown to decrease CMRO2 with minimal effect on CBF in normocapnia and to decrease CMRO2 and CBF in response to norepinephrine infusion. Hervonen et al documented leakage of H norepinephrine in gerbils subjected to 1 hour of carotid occlusion. Extravasation was first noted in animals sacrificed after 5 hours and was marked in animals sacrificed at 72 hours.

As propranolol is a lipophilic substance, it readily passes the blood-brain barrier and appears in high concentrations in brain. Thus, it is available for interaction with beta-adrenergic receptors on cerebral vessels, neuronal and glial membranes. Weksler et al demonstrated that propranolol inhibits platelet aggregation and serotonin release induced by adenosine diphosphate (ADP), epinephrine, collagen and thrombin. The d-isomer, which manifests little beta-blocking activity, was equally effective in preventing platelet aggregation, as the l-isomer. All platelet activities affected by propranolol were calcium dependent, suggesting that the drug interferes with calcium availability. Propranolol's membrane stabilizing effects were perviously theorized by Mills and Roberts to inhibit catecholamine-induced platelet aggregation. As abnormal platelet function has been noted in both experimental cerebral ischemia and during acute cerebral ischemia in humans and propranolol's anti-platelet activity may be beneficial in preserving the microcirculation.

The role of calcium as a mediator of cell death has recently been postulated. Propranolol may interfere with calcium influx by reducing the population of receptor operated channels and abolishing cyclic AMP-dependent protein kinase mediated phosphorylation of slow inward current channels. Propranolol also reduces calcium dependent phospholipase A2 activity in platelets and mouse lymphoma cells. The enzyme phospholipase promotes release of a number of fatty acids, including arachidonic acid, which inhibits the phospholipase A2.

### Table 2: Carbon Perfusion in the Right Cerebral Hemisphere of 30 Cats Undergoing Right Middle Cerebral Artery Occlusion

<table>
<thead>
<tr>
<th>Grade of carbon perfusion defects</th>
<th>Untreated cats</th>
<th>Propranolol treated cats</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>III</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

### Table 3: Vital Dye Extravasation in the Right Cerebral Hemisphere of 30 Cats Undergoing Right Middle Cerebral Artery Occlusion

<table>
<thead>
<tr>
<th>Fluorescein staining</th>
<th>Evans blue staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>Moderate</td>
</tr>
<tr>
<td>Untreated cats</td>
<td>2</td>
</tr>
<tr>
<td>Propranolol treated cats</td>
<td>3</td>
</tr>
</tbody>
</table>
of free fatty acids, including arachadonic acid, and has been implicated in disruption of organelle and plasma membrane structure and function.\textsuperscript{48} In addition, release of arachadonic acid increases production of potentially deleterious substances such as prostaglandins F\textsubscript{2a}, and E\textsubscript{2}, and thromboxane A\textsubscript{2}.

In addition to propranolol's effect on precursor production of the prostaglandin cascade, it has also been shown to inhibit thromboxane A\textsubscript{2} production in human platelets\textsuperscript{53} through inhibition of thromboxane synthetase.\textsuperscript{54} Prostacyclin release from endothelial cells is not affected by propranolol.\textsuperscript{55} In fact, the ability of media from cultured endothelial cells to inhibit platelet aggregation, presumably due to prostacyclin production, was greatly enhanced with propranolol.\textsuperscript{55}

Other experiments have suggested potential for enhancement of blood flow. Prostacyclin vasodilator response in rabbit isolated hearts was significantly augmented by addition of propranolol or sotolol.\textsuperscript{56} As d-propranolol possesses little beta-adrenergic blocking properties and sotolol no membrane stabilizing activity,\textsuperscript{57} the authors assumed another non beta-adrenergic effect was operative. Calcium antagonist activity was suggested as the mechanism by which propranolol inhibits contractions of isolated human basilar artery to locally applied prostaglandins,\textsuperscript{58} and contractions of isolated dog coronary artery to exogenous calcium.\textsuperscript{59}

Another membrane effect of propranolol which could potentially be beneficial in ischemia is the drug's ability to shift the oxygen hemoglobin dissociation curve to the right, thus allowing improved oxygen delivery to tissues.\textsuperscript{60} Osmotically induced erythrocyte lysis is inhibited by both d- and dl-propranolol,\textsuperscript{52} a probable non-beta stabilizing effect.

Comparison of Results of 3 Hour and 6 Hour Right MCA Occlusion Models.

Racemic propranolol and its d-isomer manifested a protective effect in cats subjected to 3 hours of right MCA occlusion.\textsuperscript{7} Light microscopic findings disclosed a significant reduction in size of infarcts in the d-l-propranolol and d-propranolol treated group as compared with untreated controls. In the current 6 hour right MCA occlusion study, however, no statistically significant difference in infarct size was noted.

Macroscopic findings were similar in both 3 and 6 hour studies. Carbon filling defects tended to be less severe in propranolol treated cats, but a significant difference was not achieved. This trend was also apparent in blood-brain barrier changes with untreated cats showing more Evans blue extravasation. Reduced Evans blue leakage suggested that propranolol might act on endothelial cells in protecting the blood-brain barrier. Johansson et al\textsuperscript{61} found that propranolol reduced Evans blue extravasation in rats subjected to barrier disruption by epinephrine induced blood pressure elevation. There was no protection with d-propranolol, suggesting a beta-adrenergic mechanism.

Regional cerebral blood flow was not adversely affected in 3 hour or 6 hour occlusion propranolol treated cats. Other investigators have reported minimal or no effect\textsuperscript{15} or a deleterious effect\textsuperscript{2-24} of propranolol on cerebral blood flow. Studies have differed however as to species, route of administration, dosage, and modes of measurement.

Although treatment with propranolol has theoretical benefits, the results of the present middle cerebral artery occlusion study did not indicate significant protection. Further experiments utilizing larger doses of racemic propranolol and more intensive investigation of the d-isomer will be necessary to define the roles of propranolol, if any, in the treatment of cerebral ischemia.

Acknowledgments

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