Isoproterenol and Propranolol: Ability to Cross the Blood-Brain Barrier and Effects on Cerebral Circulation in Man

JES OLESEN, M.D., KJELD HOUGÅRD, M.D., AND MARIANNE HERTZ, M.D.

SUMMARY Using the "double indicator" technique the ability of 3H-isoproterenol and 14C-propranolol to cross the blood-brain barrier was studied in man. In 3 subjects extraction of isoproterenol was 3.8% in a single passage and the PS product was 2.0 ml/100g/min. In 4 patients extraction of propranolol was 63% and PS was 46.7 ml/100/min. Regional cerebral blood flow (rCBF) was studied in man with the 133Xe-intraarterial injection method. Intracarotid isoproterenol (3 μg/min, 6 patients) caused a significant reduction in rCBF, but after correction for a concomitant decrease in arterial PCO2 the alteration was no longer significant (59.8 — 51.7/57.4 ml/100g/min.). Intracarotid propranolol (0.15 mg/kg, 11 patients) caused no significant change in rCBF, but after correction for arterial PCO2 change the alteration although only 4% was just significant, p < 0.05. (56.3—55.8/54.1 ml/100g/min.). After propranolol the rCBF changes caused by alterations in the arterial PCO2 were normal and the focal flow increase during hand work could not be changed by simultaneous intracarotid propranolol.

RECENT scientific investigations have dealt with the effects of cervical sympathetic nerve stimulation/section or with the pharmacology of the cerebrovascular α-adrenergic receptor mechanisms. This extensive literature is somewhat contradictory, but increasing evidence supports the contention that sympathetic nerves via α-receptors play a significant role in the tone of brain vessels under certain circumstances. For the most recent studies see Edvinsson and McKenzie. Much less is known about the significance of β-adrenergic receptors on brain vessels, and, again, the literature is contradictory.

The aim of the present investigation was to study the effect of β-adrenergic stimulation with isoproterenol and blockade with propranolol on the regional cerebral blood flow in man. To better evaluate these results, the ability of isoproterenol and propranolol to cross the blood-brain barrier was also measured.

Material and Methods

All subjects were in-patients of the neurological department, in whom a diagnostic carotid arteriogram had been ordered by the responsible neurologist. Patients with obvious brain tumors and large strokes as well as patients with heart disease were excluded. Most of the patients suffered from persistent unilateral headaches, epilepsy or dementia, and most of the angiograms turned out to be normal. Informed consent was obtained in all cases.

The studies were performed in the morning. No premedication was given and the patients were fasting. For the CBF studies a catheter was placed percutaneously into the internal carotid artery under local anesthesia (lidocaine 1% without noradrenaline). For blood-brain barrier studies a catheter was inserted into the bulb of the internal jugular vein after percutaneous puncture in the neck.

Regional cerebral blood flow was measured with the intraarterial 133Xe injection technique utilizing either a 16 or a 35 detector system. The RCBF was calculated from the initial slope of the first 1—2 minutes of the logarithmically displayed clearance curves (CBF_initial). Systolic, diastolic and mean intraarterial blood pressure in the internal carotid artery were measured with a transducer, and, in conjunction with each flow determination, a sample was drawn from the carotid catheter for determination of arterial Pco2 (Paco2). The flow during drug infusion was corrected for small differences in Paco2 with 4% per mm Hg difference in Paco2. The cerebrovascular reactivity to changes in Paco2 was calculated as

\[ \Delta \log \text{CBF} \div \Delta \text{Paco2} \]

according to Olesen et al.

Calculations of statistical significance were performed with Wilcoxon's rank sum test for paired observations. Blood-brain barrier studies were performed with the indicator diffusion method of Crane as modified for human use by Lassen et al. and Bolwig et al. (fig. 1). In brief, 3 ml of a mixture of 24Na+ (30 μCi, New England Nuclear Corporation) and 14C-propranolol,† (15 μCi) or 3H-isoproterenol (30 μCi, Rishøj) and 1H-isoproterenol (50 μCi, Amersham) was injected as a bolus into the internal carotid artery. Simultaneously, a continuous series of blood samples of 1.0 ml each was collected from the jugular catheter into dry heparinized tubes by means of a sampling machine with a speed of 1 sample per second. A reference solution was obtained by adding injectate to heparinized venous blood drawn immediately before the injection so that the injectate could be analysed under the same conditions as the venous samples. After centrifugation 300 μl of plasma from each sample was mixed with 15 ml of scintillation fluid (Instagel, Packard). 24Na+ content was determined in a crystal

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*Further clinical data may be obtained from the author.

†Kindly supplied by Imperial Chemical Industries (ICI).
scintillation counter (Packard Autogamma 5358), and after decay of $^{24}$Na$^+$, $^{14}$C and $^{36}$Cl in the samples were counted in a liquid scintillation counter (Packard Tricarb 3375), and corrections were applied for quench and channel spillover using the method of external standardization. The concentrations of the blood samples were expressed in relative units by division with the corresponding concentrations in the reference solution.

The extraction (E), i.e. the loss of test substance in a single passage relative to the intravascular co-tracer, was determined as

$$E = \frac{A_{\text{ref}} - A_{\text{test}}}{A_{\text{ref}}}$$

(1)

where $A_{\text{ref}}$ and $A_{\text{test}}$ are the areas under the upstroke part of the venous outflow curves for reference and test substances respectively (fig. 1). The permeability - surface area product (PS) was determined as

$$PS = -F \times \ln(1 - E)$$

(2)

where $P$ is the fractional amount of substance that passes the capillary wall per unit area and unit time and $S$ the capillary surface area. $F$ is the blood flow. PS is equivalent to the capillary diffusion capacity (CDC).

**BLOOD-BRAIN BARRIER IN MAN**

**Figure 1. Illustration of the "double indicator" technique for blood-brain barrier studies in man. A mixture of tracer and co-tracer is injected as a bolus intracarotidly and blood samples 1/second are simultaneously drawn from the carotid artery and the bulb of the internal jugular vein. In the upper right corner the concentration curves for tracer (broken line) and co-tracer (full line) are plotted as a function of time. The loss of test substance in a single passage relative to the intravascular co-tracer ($E$) and the capillary diffusion capacity ($CDC = PS$ product) can be calculated.**

To minimize the effect of intravascular phenomena $^{24}$Na$^+$ was used as co-tracer for isoproterenol because none of these substances crosses the erythrocyte membrane, and $^{36}$Cl$^-$ was used as a co-tracer for propranolol because both of these do pass into the erythrocyte.

For the study of isoproterenol we used a solution of 1 mg/ml prepared by our pharmacy without the use of additives. It was diluted in physiological saline immediately before the study. rCBF was measured in the resting state, then the effective isoproterenol dose was determined by slowly increasing the rate of infusion into the internal carotid artery until a significant increase in heart rate, not exceeding 100 beats per minute, was produced. This infusion rate was continued for 4 minutes prior to the $^{133}$Xe injection and continued during the 2 minutes recording period. After 15 minutes rCBF was measured again in the resting state. In table 2 the second resting value was used for comparison with isoproterenol.

For the study of propranolol we used a commercially available preparation of 1 mg/ml propranolol hydrochloride (Inderal) diluted in physiological saline immediately before the study. rCBF was measured twice in the resting state. Then over the next 10 minutes propranolol 0.15 mg/kg total dose was infused and rCBF measured immediately after termination of the infusion. After another 15 minutes rCBF was again measured during alterations of Paco2 either by breathing an air mixture of 7% CO2 and 93% O2 or by voluntary hyperventilation. After these procedures, carotid arteriography was performed through the internal carotid artery catheter. No complications were encountered.

**Results**

Results of the blood-brain barrier investigations of isoproterenol in 3 patients and of propranolol in 4 patients are listed in table 1. Values are in each case the average of 2 independent determinations.

Mean extraction of isoproterenol in a single passage of the brain circulation was 3.8% and the calculated PS product was 2.0 ml/100g/min. The mean extraction of propranolol was 63.0% and the mean PS product 46.7 ml/100 g/min. The effect of intracarotid isoproterenol on rCBF was studied in 6 patients (table
2). The arterial mean blood pressure was unchanged, but the heart rate increased from 71 to 94 b/min ($p = 0.02$). The arterial PCO$_2$ decreased from 38.8 mm Hg to 36.1 mm Hg ($p = 0.02$). The many regional CBF values measured from each hemisphere showed the same response to isoproterenol and to propranolol in all patients, as judged from visual comparisons of the flow curves. Therefore, only hemispheric average values are considered in the following. The resting state mean rCBF in the 6 patients was 59.8 ml/100 g/min and during isoproterenol 51.7 ml/100 g/min. When, however, correction for the induced change in PCO$_2$ was carried out, mean rCBF during isoproterenol was 57.4 ml/100 g/min. The uncorrected value was lower than the resting value ($p = 0.02$) but the corrected value was not significantly different. The rCBF during isoproterenol varied more than we usually see in repeated studies, but in either direction. This may correspond to a certain feeling of unrest and anxiety felt by several patients during isoproterenol. Otherwise, no untoward effects were encountered.

The effect of propranolol was studied in 11 patients (table 3, fig. 2). There was no change in mean cerebral blood pressure, but the heart rate decreased from 77 to 68 b/min ($p < 0.01$). The arterial PCO$_2$ was not significantly changed. The rCBF at rest was 56.3 ml/100 g/min and during propranolol 55.8 ml/100 g/min, which was not significantly different. When correction was carried out for the small difference in PCO$_2$, the propranolol flow value decreased to 54.1 ml/100 g/min. This was significant ($p < 0.05$).

During a change in arterial carbon dioxide tension by 7% CO$_2$ breathing in 5 patients and voluntary hyperventilation in 3 patients 15 minutes after termination of the propranolol infusion, the CBF changed appropriately. The calculated vasoreactivity to PCO$_2$ changes was $\Delta \log \text{CBF} \over \Delta \text{PCO}_2 = 0.180$ which corresponds to 5.1% change in CBF per mm Hg change in PCO$_2$. This is slightly higher than previously reported in patients who did not receive propranolol, but not significantly so.

To further elucidate, if beta adrenergic receptors might play a role under alterations of physiological functions, rCBF was measured in the resting state and after intracarotid propranolol 0.15 mg/kg combined with arm work in one patient. The usual focal increase in rCBF corresponding to the sensory-motor hand area was seen.

## Table 2. Effect of Isoproterenol on Regional Cerebral Blood Flow in Man

<table>
<thead>
<tr>
<th>Age/sex</th>
<th>(Dose) (micrograms/min)</th>
<th>MABP (mm Hg)</th>
<th>Heart rate (beats/sec)</th>
<th>Paco$_2$ (mm Hg)</th>
<th>Mean hemispheric rCBF (ml/100 g/min)</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td>isoproterenol</td>
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<tr>
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<td>$P = 0.02$</td>
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</table>

## Table 3. Effect of Intracarotid Propranolol on Regional Cerebral Blood Flow in Man

<table>
<thead>
<tr>
<th>Age/sex</th>
<th>MABP (mm Hg)</th>
<th>Heart rate (beats/min)</th>
<th>Paco$_2$ (mm Hg)</th>
<th>Mean hemispheric rCBF (ml/100 g/min)</th>
<th>CBF reactivity to Paco$_2$ change*</th>
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<tr>
<td>68 M</td>
<td>68</td>
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<tr>
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</table>

*Calculated as $\Delta \log \text{CBF} \over \Delta \text{Paco}_2$.

These two patients had intravenous infusion. The others intracarotid.
Discussion

Passage of Isoproterenol and Propranolol Across Blood-Brain Barrier

No data are available in the literature concerning the ability of isoproterenol to cross the blood-brain barrier. From the hydrophilic nature of the molecule one might expect diffusion to be very slow, but the possibility of active uptake mechanisms still existed. The extraction of 3.8% found in the present study corresponds to that of sodium or other hydrophilic molecules. It is likely that a significant part of this extraction stems from areas known to be devoid of a blood-brain barrier. The extraction is clearly much smaller than that seen for amino acids and other substances that pass the barrier by facilitated diffusion. During prolonged infusion of high doses, such as in the present study, an extraction of 3.8% might, however, possibly create rather high concentrations of isoproterenol in and around the brain resistance vessels. Autoradiographic studies with scanning devices would be necessary to answer this question.

The lipophilic nature of the propranolol molecule indicates that it should easily pass the blood-brain barrier. C-propranolol has been found in significant amounts in the brain a few minutes after an intravenous injection. The present study is the first quantification of propranolol's ability to pass the blood-brain barrier. About 2/3 of propranolol in the blood crosses the blood-brain barrier in a single passage through the brain. This is almost the same extraction as for water. Propranolol injected into the carotid artery or intravenously is thus almost immediately present in the brain. By analyzing the down-slope of the propranolol curve (fig. 1) it was apparent that propranolol was washed out of the brain quickly. There was no indication of strong binding of propranolol to specific receptors like, for example, digitalis in the heart.

Effect of Isoproterenol and Propranolol on Cerebral Blood Flow and Cerebrovascular Reactivity

Previous Studies of Local Vascular Reactions

Edvinsson and Owman recorded the reactions of the isolated middle cerebral artery of the cat in an organ bath during isometric conditions. They found only minor relaxing effects of low doses of isoproterenol and terbutaline but a pronounced constriction at high doses. On the other hand, when the artery was given an increased tone by 5-hydroxytryptamine, both β-adrenergic stimulants caused it to relax although isoproterenol was much more powerful than terbutaline. This response could be competitively inhibited by propranolol and N-isopropyl-p-nitrophenylethanolamine. Edvinsson and Owman, therefore, concluded that the brain arteries possess β-adrenergic receptors of the β- subtype. On the other hand, Melamed et al., using a fluorescent marker for β-adrenergic receptor sites, were unable to demonstrate such receptor in rat pial and intraparenchymal brain vessels. This is in contrast to other tissues where β-adrenergic receptors could easily be demonstrated with the same technique. Micropipette studies of the pial arterial reactions in vivo also failed to demonstrate any significant β-adrenergic influence on vascular diameter. Over a wide concentration range which corresponds to the concentrations used by Edvinsson and Owman, no change in arteriolar diameter was caused by propranolol. With 2.5 × 10⁻⁴ dilatations of 4–5%, and with 2.5 × 10⁻⁴ and 1.25 × 10⁻³ constrictions of 3–5% were seen. Similar results were found in other in vivo studies of pial arteriolar reactions.

Previous Studies of Effect of Isoproterenol and Propranolol on Cerebral Blood Flow

In animal experimental studies varying results have been obtained. In a dog preparation, isoproterenol caused a marked increase in CBF and CMRO₂ and propranolol caused reduced CBF with unaltered CMRO₂, but these results were almost certainly affected by extracranial blood contamination. In other dog experiments — also open to technical criticism — a CBF increase was seen with isoproterenol. In another study local blood flow was qualitatively estimated with thermistors in the caudate nucleus and the lateral geniculate body of rabbits. Isoproterenol caused marked increases in caudate blood flow, but not in the lateral geniculate body flow. Most previous studies show no effect of propranolol on CBF or CMRO₂. McKenzie et al. found CBF unaltered but CMRO₂ reduced by propranolol. In man the acute effect of propranolol on hemispheric blood flow of stroke patients has been.

![ACUTE EFFECT OF INTRACAROTID PROPRANOLOL](attachment:image.png)

Figure 2. The lack of effect of propranolol on the regional cerebral blood in man is demonstrated. Values are not corrected for a small difference in arterial Pco₂.
 studied. The strokes must have been very severe since the resting state hemispheric blood flow was very low (34 ml/100 g/min). Approximately 2 mg of propranolol was infused into the carotid artery over a 15–20 minute period. Fifteen minutes later CBF and CMRO₂ were found to be moderately but significantly lower than control. It is, however, uncertain if this is a drug effect, since the dosage was too low to ensure systemic β-blockade. Response to changes in Paco₂ and to decreases in perfusion pressure were normal after propranolol, but the autoregulatory response to increased perfusion pressure improved. In our opinion the latter finding must be interpreted with caution. We have often noted that in patients with severe brain disease autoregulation may vary on repeated testing. The chronic effects of propranolol were evaluated by Strandgård. Hypertensive patients treated with propranolol had normal autoregulation.

Present Results

It is well known that isoproterenol, like noradrenaline, usually induces a slight hyperventilation as also can be seen in the present study. It is, therefore, clear that the CBF result must be corrected for changes in Paco₂. Thus corrected the CBF was unaltered by isoproterenol. This is in accordance with the low ability of isoproterenol to cross the blood-brain barrier and with the finding of only minor changes in pial arteriolar diameter with isoproterenol. Since our flow method is relatively insensitive to deeper brain structures, there is no real discrepancy between our findings and those of Sercombe et al., who found caudate nucleus flow to increase with isoproterenol.

Our propranolol data demonstrate a rapid passage across the blood-brain barrier. Therefore, we infer that the high doses infused must have caused a complete β-adrenergic blockade of receptors in blood vessels and brain. The CBF decrease, although statistically significant, was only 4% corresponding to a 1 mm Hg change in Paco₂. Recalling the well known sedative effect of propranolol but possibly not enough to block complete β-adrenergic blockade of receptors in blood vessels and brain, this may explain the difference between our findings and those of McKenzie. Finally, we found that intracarotid propranolol did not block the focal rCBF increase induced by hand work.

Concluding Remarks

The present investigation as well as the existing literature are in agreement with the following synthesis: 1) The cervical sympathetic nerves activate α-receptors in the cerebral vessels and these α-receptors can be stimulated/blocked by appropriate drugs. In primates the effect is weak and probably confined largely to the “inflow tract.” 2) No beta-adrenergic peripheral innervation of brain vessels has been found so far. 3) Noradrenergic fibers from the brain stem project to other brain areas and stimulation of these fibers increases cerebral blood flow via β-adrenergic receptors. During blood-brain barrier disruption circulating catecholamines may also increase cerebral blood flow and metabolism by stimulation of β-adrenergic receptors — presumably the same. 4) The cerebral blood flow increase caused by beta-adrenergic stimulation is associated with an increase in cerebral oxygen consumption. It is, therefore, not an isolated vascular reaction, but the flow increase is, rather, secondary to metabolic activation. Since a close coupling between metabolism and flow is known to exist in many other situations, the findings do not necessarily indicate existence of beta-adrenergic receptors in the cerebral vascular walls.

References

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Effects of Acute Hypertension on Brain Metabolism in Normotensive, Renovascular Hypertensive and Spontaneously Hypertensive Rats

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SUMMARY Effects of angiotensin-induced acute hypertension on cerebral metabolism were studied in normotensive (NTR), spontaneously hypertensive (SHR) and experimental renovascular hypertensive rats (RHR).

Lactate, pyruvate and adenosine triphosphate (ATP) concentrations in the brain frozen in situ at 18-20 min after angiotensin infusion, which raised mean arterial pressure (MAP) by 28-62% of control, were determined by enzymatic methods. Supratentorial lactate was significantly increased to 135% of control in RHR, its increase being correlated with the degree of hypertension, whereas it remained unchanged in NTR or SHR. Furthermore, RHR showed a tendency toward increase in lactate/pyruvate ratio with a decrease in ATP despite no change of arterial acid-base balance measured simultaneously before and after acute induced hypertension.

From the present study, it is postulated that some renal factor seems to contribute ischemic metabolic changes in RHR following acute hypertension. The possible effect of renin on the vascular permeability is discussed as the pathogenesis of hypertensive encephalopathy.

THE PATHOGENESIS of hypertensive encephalopathy has not been fully understood. There have been 2 major contradictory theories proposed, 1 of which is intense arterial spasm in response to the rise in blood pressure with reduction of flow into the capillary bed resulting in ischemia, increased capillary permeability and cerebral edema. This was originally proposed by Oppenheimer and Fishberg, and was supported by Byrom's in vivo observations of the pial circulation of renovascular hypertensive rats.

The breakthrough or failure of autoregulation is another theory, which was recently proposed by Scandinavian investigators. They have demonstrated that breakthrough of the cerebral blood flow autoregulation occurs when blood pressure rises beyond a certain upper limit, resulting in an increase in cere-
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