Duration versus degree of hypotension as critical conditions of brain infarction in the albino rat

RUDOLF LAAS, M.D., BERND JAHN, M.D., AND KAY KESSLER, M.D.

Department of Neuropathology, Eppendorf Hospitals, University of Hamburg, Hamburg, Federal Republic of Germany

In 41 anesthetized, spontaneously breathing male adult albino rats, cerebral hypotension of precisely defined duration and magnitude was induced by means of controlled arterial hemorrhage. One common carotid artery was occluded throughout the hypotensive period, and the target pressure was monitored in the ipsilateral internal carotid artery. Regional brain infarcts developed in all 16 animals with a target pressure of 14 mm Hg maintained for 90 minutes and in all five animals with a target pressure of 12 mm Hg maintained for 70 minutes. However, the brains of all 10 rats with a target pressure of 17 mm Hg maintained for 80 minutes remained intact. In two further groups of five animals each with target pressures of 15 mm Hg for 80 minutes and 16 mm Hg for 90 minutes the incidence of infarct was about 30%. There were no marked differences between the five groups of rats in body weight, body temperature, heart rate, respiratory rate, PaO₂, PaCO₂, arterial pH, or hematocrit.

The data suggest that, in the rat, the clear-cut threshold for the induction of brain infarcts is a function of the severity and duration of arterial hypotension. Evidence is presented indicating that this function is distinctly species-dependent, due to species differences in the dilatory capacity of the arteries supplying the brain rather than species differences in brain vulnerability.

KEY WORDS • cerebral infarction • hypotension • rat

Blood flow is a relevant parameter of the development of cerebral infarction. However, methods currently available for the measurement of cerebral blood flow (CBF) are not sufficiently precise for use in such a study. We have previously shown that blood pressure, which can be measured reliably, is also a valid indicator of the outcome of cerebral ischemia in the Mongolian gerbil. These observations confirm results obtained from monkeys and from cats.

In this paper, we report the accuracy with which regional brain infarcts can be ascribed to degree and duration of hypotension when other relevant parameters are kept close to normal levels. We used the experimental model described by Selkoe and Myers with one difference: in order to avoid hypotensive cardiac failure, cephaliac blood pressure was lowered not only by systemic hypotension but also by additional unilateral carotid ligation.

Materials and Methods

The 41 male albino rats (Cbb:THOM) used in this study had had free access to rat chow and tap water. Body weight ranged between 460 and 520 gm. The animals were anesthetized by intraperitoneal injection of ketamine (125 mg/kg body weight) and placed in a supine position. A plastic catheter was introduced into the left femoral vein for intravenous injections. A second catheter was inserted retrogradely into the left femoral artery and connected by a three-way stopcock to a Statham pressure transducer* for the continuous measurement of systemic arterial blood pressure. For the temporary extravasation of arterial blood, a vertical tube was connected to the stopcock and to a 20-ml syringe which acted as a reservoir. The height of this reservoir could be varied in order to control the rate of extravasation and reinfusion of arterial blood, respectively.

A third catheter was inserted retrogradely into the right external carotid artery to monitor the blood pressure in the internal carotid artery. It was connected by a three-way stopcock to a second Statham pressure transducer and to a vertical tube containing heparinized saline for calibration purposes. Both transducers were connected to amplifiers and to a recorder.

* Pressure transducer, Model P 50, manufactured by Statham Laboratories, Inc., Hato Rey, Puerto Rico.
Body temperature was monitored continuously by a rectal probe and was kept constant at 36.5° to 37.5°C by use of a heating pad. Heparin was given at a dose of 200 IU/kg body weight. Both pressure transducers were adjusted to the atrial level.

Before hypotension was induced by removal of arterial blood, the right common carotid artery was reversibly occluded with a loop of 3-0 surgical thread. The occlusion reduced the arterial blood pressure in the ipsilateral internal carotid artery by about 50%. The duration of the entire procedure up to this point was about 50 minutes. Arterial blood was removed via the vertical tube connected to the femoral artery catheter. The rate of blood pressure decline was closely controlled so that the preselected target pressure in the right internal carotid artery was reached within 6 minutes. The schedule of the target pressures and the durations of hypotension are shown in Table 1. Hypotension was maintained as near as possible to within ± 2 mm Hg of the preselected target pressure by lifting and lowering the reservoir. The retained blood was kept at a mean temperature of 37°C by winding a plastic tube rinsed by heated water around the reservoir.

Arterial blood gases, arterial pH, and hematocrit were measured at 15-minute intervals throughout the hypotensive period, immediately after termination of hypotension, and 20 minutes later. A decrease of pH below 7.4 was compensated for immediately by intravenous injection of sodium bicarbonate (1-M solution). Anesthesia was maintained by intravenous injection of 2 mg of ketamine at 10-minute intervals. The animals breathed spontaneously throughout the entire procedure.

Five minutes before termination of hypotension, the rats received 0.8-ml intravenous injections of digitoxin (0.04 μg/kg body weight) for cardiac protection and 1 ml Evans blue dye in 1% saline for visualization of possible breakdown of the blood-brain barrier. Cerebral hypotension was terminated by reopening the right common carotid artery and by intra-arterial reinfusion of the blood volume withdrawn during the hypotensive period. Heparin was reversed by the slow intravenous injection of protamine sulfate. The catheters were then removed and the wounds closed. The animals were observed for about 6 hours after recovery from anesthesia.

At least 10 days after induction of hypotension, the brains were perfusion-fixed by injection of 10% formalin containing 1% acetic acid into the ascending aorta. Four hours after perfusion, the brains were dissected from the skull and the pattern of Evans blue dye extravasation was recorded. The brains were embedded in Paraplast and cut in two planes. One was through the rostral striatum and the other through the largest extent of the thalamus. Sections 10 μm thick were stained with cresyl violet and the van Gieson technique.

Systemic arterial blood pressure and blood pressure in the right internal carotid artery obtained at 1-minute intervals were analyzed statistically.

### Results

All animals survived the hypotensive period. Symptoms indicative of transient cardiac failure were not observed. The standard deviation of the target pressures in 24 of the 41 animals was ± 1.0 mm Hg or below, and, except for one case, did not exceed ± 2.0 mm Hg.

All animals with hypotension of 12 mm Hg for 70 minutes and 14 mm Hg for 90 minutes developed regional brain infarcts. The infarct incidence was lower in the animals with hypotension of 15 mm Hg for 80 minutes and 16 mm Hg for 90 minutes, and the brains of all animals with hypotension of 17 mm Hg for 80 minutes remained intact (Table 2). In the three groups with lesions, the pattern was similar. The lesions were small to medium in size, and their volume did not exceed a quarter of the affected hemisphere. All animals developed infarcts in the basal ganglia, and six animals each had cortical infarcts in the convexity of the forebrain and/or in Ammon's horn.

During the hypotensive period there were no significant differences in mean heart rate, PaCO₂, PaO₂, arterial pH, and hematocrit.† Arterial pO₂ was kept well above 100 mm Hg and arterial pH above 7.3 in all animals (Fig. 1). There was a slight although insignificant transient hypercapnia in the group with hypotension of 12 mm Hg for 70 minutes (Fig. 2); this group also showed a significant depression of the mean arterial blood pressure at the 20th minute into recovery — a finding seen to a lesser degree in the group with hypotension of 14 mm Hg for 90 minutes.

During the hypotensive period, the hematocrit and respiratory rate declined slightly. In most animals, the systemic arterial blood pressure had to be further reduced during the hypotensive period to compensate for the effects of gradual opening of collateral vessels. The main alteration in behavior during the post-hypotensive period consisted in tight circling movements. This so-called "rotational behavior" occurred in 18 of the 24 rats that developed brain infarcts and in none of those with intact brains. Fifteen rats circled ipsiversively and three contraversively in relation to the infarct.

### Table 1

Severity and duration of hypotension in 41 rats

<table>
<thead>
<tr>
<th>Target Pressure</th>
<th>Duration of Hypotension</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>70 Min</td>
</tr>
<tr>
<td>12 mm Hg</td>
<td>5</td>
</tr>
<tr>
<td>14 mm Hg</td>
<td>—</td>
</tr>
<tr>
<td>15 mm Hg</td>
<td>—</td>
</tr>
<tr>
<td>16 mm Hg</td>
<td>—</td>
</tr>
<tr>
<td>17 mm Hg</td>
<td>—</td>
</tr>
</tbody>
</table>

† Absolute measures of mean arterial pressure, heart rate, respiratory rate, PaCO₂, arterial pH, and hematocrit can be obtained from the authors on request.

R. Laas, B. Jahn and K. Kessler
Hypotension and brain infarct in rats

FIG. 1. Changes in mean arterial blood pressure (MABP), heart rate, and respiratory rate before, during, and at 20 minutes after the hypotensive period. The data are given as mean and standard error of the mean. In the designation of study groups, the first number indicates the target pressure (in mm Hg) and the second the duration of hypotension (in minutes).

Discussion

Our data show that the rat has a narrow threshold for the induction of regional brain infarcts as defined by arterial blood pressure and time. Small increases of the target pressure by 1 to 3 mm Hg and/or reductions of the duration by 10 minutes were shown to ameliorate outcome considerably (Fig. 3).

The data appear to be quite reliable and valid since in 24 of the 41 rats more than 96% of the 1-minute values were within the target pressure ± 2 mm Hg. Furthermore, there were only insignificant differences in the recorded and/or controlled parameters between the study groups (Fig. 2).

The moderate hypercapnia that occurred during the first 45 minutes of hypotension at 12 mm Hg for 70 minutes is not likely to have worsened outcome considerably in that group, since vascular CO₂ reactivity is known to be abolished in states of severe hypotension and since pH was kept in the normal range. The steep increase of hematocrit during the recovery period confirms the findings reported by others in dogs.

These data allow preliminary insight into the relationship between the degree and duration of hypotension, and the role of both, in the development of regional brain infarcts. Two points were demonstrated on the hypothetical curve of critical values (namely, 14 mm Hg and 90 minutes, and 12 mm Hg and 70 minutes). A third point may be defined by the critical duration which is related to total ischemia (that is, about 10 to 15 minutes).

The end-point of the curve is likely to approach an asymptote, being defined by the lowest arterial blood pressure that is tolerated for a theoretically infinite duration. For gerbils with ligation of one common carotid artery, this asymptote was found to lie at a common carotid artery stump pressure of about 30 mm Hg. As outlined in Fig. 3, this value may also apply to the rat. The curve was validated further. By interpolation it was possible to determine another pair of critical values for the induction of regional brain infarcts (that is, 10 mm Hg and 40 minutes).

TABLE 2

Incidences of regional brain infarct in rats in the five groups

<table>
<thead>
<tr>
<th>Postmortem Findings</th>
<th>Hypotension Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 mm Hg</td>
</tr>
<tr>
<td></td>
<td>70 min</td>
</tr>
<tr>
<td>brain infarct</td>
<td>5</td>
</tr>
<tr>
<td>intact brain</td>
<td>0</td>
</tr>
<tr>
<td>total</td>
<td>5</td>
</tr>
</tbody>
</table>

FIG. 2. Changes in hematocrit, pH, and arterial pCO₂ during and at 20 minutes after the hypotensive period. The data are given as mean and standard error of the mean. In the designation of study groups, the first number indicates the target pressure (in mm Hg) and the second the duration of hypotension (in minutes).
The concept of a functional blood pressure-time threshold for the development of regional brain infarcts modifies the assumption of a well-defined lower blood pressure threshold with a minimal time demand.\textsuperscript{2,12,28} This assumption was based on data which now may be reinterpreted. Brierley, \textit{et al.},\textsuperscript{2} produced severe hypotension in 15 rhesus monkeys by means of hemorrhage, trimethaphan injections, and head-up tilting. Six animals developed mild and four had severe regional brain infarcts. As depicted in our Fig. 3, the coordinates of the pressure and time values of the affected animals appear to fit into a curve that is similar but not identical to that found for the rat. It agrees with our concept that the curve of parameters in those animals that developed severe lesions has seemingly shifted to the left and the curve of five animals with intact brains to the right. The two animals that did not obey this rule (marked with asterisks in Fig. 3) were probably protected by deeper pentobarbital anesthesia in one and by irregular onset of hypotension in the other.

Selkoe and Myers\textsuperscript{28} found critical coordinates of about 20 mm Hg and 30 minutes as based on measurements in six \textit{macaca mulatta} monkeys. Values of two other monkeys with infarcts deviated considerably from this level (25 mm Hg and 65 minutes, and 31 mm Hg and 75 minutes). Presuming that these values are also representative, again a critical curve can be estimated (Fig. 3).

Although the findings in both studies were obtained from monkeys, the slope of the curve constructed from the data given by Brierley and coworkers\textsuperscript{2} is steeper than that estimated from the data of Selkoe and Myers.\textsuperscript{28} This discrepancy may indicate that the critical pressure-time curve is even strain-dependent; however, it may be better explained by the considerable differences in the experimental conditions. In contrast to Selkoe and Myers, Brierley and coworkers used artificial respiration and head-up tilting and failed to compensate for acidosis. Furthermore, these authors determined cerebral perfusion pressure. Thus, in order to regain the original arterial blood pressure values, we had to subtract the venous sinus pressure which was reported to reach a level of about \(-7\) mm Hg during the hypotensive period.

Dong, \textit{et al.},\textsuperscript{3} were not able to induce regional brain infarcts in dogs using the same degree of hypotension that was applied to the monkeys by Selkoe and Myers,\textsuperscript{28} even when the duration was prolonged to 60 minutes. Acidosis was compensated for but, in contrast to the monkeys, the dogs were ventilated artificially throughout the session and not just when necessary. Thus, the data presented suggest that the cerebral resistance against arterial hypotension depends on species or perhaps even on strain within a species. On the other hand, the results of our investigation indicate that, within a defined species or strain, the variation of this cerebral resistance is minimal.

This species dependence cannot be explained by dif-

![Graph](https://via.placeholder.com/150)

**FIG. 3.** Duration versus degree of hypotension in animals with intact brains and in animals with moderate or severe brain lesions based on 41 rats (this study), 15 monkeys (m. rhesus, Brierley, \textit{et al.}), and 14 monkeys (m. mulatta, Selkoe and Myers). The dotted lines represent the hypothetical curves constructed by visual estimation. Asterisks indicate two animals that did not fit into the concept proposed by the authors (see text). Art. BP = arterial blood pressure; les. = lesions.
Hypotension and brain infarct in rats

Significantly from that detected with a rat model where corresponds to those in Fig. 3.

Measurements of regional CBF after ligation of one artery and pericellular microflow in single cortical neurons of the cat and on data of others obtained by microelectrode impalements. Indeed, this curve corresponds to those in Fig. 3.

The lesion pattern found in our study did not differ significantly from that detected with a rat model where ischemic lesions were induced by a combination of ligation of one common carotid artery and hemorrhagic hypotension. It also resembled the lesion pattern found with a modified Levine preparation and with the gerbil stroke model. The close correlation between infarct development and the occurrence of circling behavior confirms data presented in previous papers.

This behavioral disturbance is thought to be due to lesioning of the ipsilateral dopaminergic striatal system. Again this symptom proved to be a reliable indicator of brain infarction. It is possible that this is also true for the circulatory depression which was demonstrated at the 20th minute into recovery in the groups with hypotension at 12 mm Hg for 70 minutes and at 14 mm Hg for 90 minutes. This phenomenon confirms findings obtained with monkeys and with the gerbil stroke model. It is thought to be due to a central mechanism which is still poorly understood and appears to be related to the extent of the infarcted area.

Conclusions

Our findings show that a threshold for brain infarction defined by blood pressure alone does not exist. At best, the value of the putative asymptote of the pressure-time curve may be regarded as a blood pressure threshold. It is further shown that this value and the shape of the critical pressure-time curves are distinctly species-dependent.

The combination of lowered blood pressure and defined duration provides a valid and reproducible model for the study of the pathophysiology of brain infarction. In contrast to CBF, blood pressure does not reflect the all-important perfusion rate of the tissue. However, this disadvantage is at present outweighed by the superior reliability of pressure measurements.

References

8. Heistad DD, Marcus ML, Abboud FM: Role of large

Manuscript received January 7, 1986.