Effect of nephrectomy and captopril on autoregulation of cerebral blood flow in rats

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Pedersen, Trine Fischer, Olaf B. Paulson, Arne Høj Nielsen, and Svend Strandgaard. Effect of nephrectomy and captopril on autoregulation of cerebral blood flow in rats. Am J Physiol Heart Circ Physiol 285: H1097–H1104, 2003.—The present study investigated the effect of circulating versus locally present renin on cerebral blood flow (CBF) and its autoregulation in rats. CBF was measured repetitively with the intracarotid ¹³⁵Xe injection method, whereas blood pressure was lowered to determine the lower limit of autoregulation. To remove renin from the blood, rats were bilaterally nephrectomized and kept alive with peritoneal dialysis for 48 h. Five groups of animals were studied: 1) nephrectomized dialyzed rats, 2) nephrectomized dialyzed rats given a single intravenous dose of the angiotensin-converting enzyme inhibitor captopril (10 mg/kg), 3) sham nephrectomized and dialyzed rats, 4) rats receiving drugs as dialyzed rats but no surgery, and 5) rats given the same diet as the other groups but no drugs and no surgery. Baseline blood pressure was significantly lower in nephrectomized rats compared with controls. Nephrectomy, captopril, sham operation, or dialysis did not influence baseline CBF. The lower limit of CBF autoregulation was significantly lower in nephrectomized rats (53 ± 4 mmHg) and sham-operated rats (58 ± 4 mmHg) rats compared with control rats (78 ± 3 mmHg). Captopril significantly decreased the lower limit in nephrectomized rats (35 ± 2 mmHg). Thus removal of circulating renin caused no change in the lower limit of autoregulation. By contrast, captopril lowered the lower limit even in the absence of circulating renin and hence appeared to exert its effect on components of the renin-angiotensin system in the cerebral resistance vessel walls.

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received no drugs and were not exposed to surgery (diet controls). In groups 1 and 3–5, eight rats underwent a 2-h time course study with repeated measurements of CBF and blood pressure, and, in each of all five groups, eight rats underwent a study of the lower level of CBF autoregulation.

**Nephrectomy and peritoneal dialysis.** Details of nephrectomy and the dialysis procedure have been described elsewhere (18). Briefly, rats were anesthetized with a 0.4 ml/kg im zoletile mixture containing 250 mg zoletile (zoletile 50; Boehringer Ingelheim Agrovet, Hellerup, Denmark), 10 ml narcocyl (xylazin 20 mg/ml; Veterinaria, Scandinavia, Skovlunde, Denmark) and 1 ml butorphanol tartrate (turbogesic, 10 mg/ml, Fort Dodge, Iowa).

The kidneys were exposed by flank incisions, fat and adrenal glands were carefully loosened and detached from the kidneys, renal vessels and ureters were ligated, and the kidneys were removed. Muscles and skin were closed separately. In sham-operated rats, the kidneys were exposed by flank incisions but not removed.

For the peritoneal dialysis, a special dialysis catheter produced in our laboratory was inserted in the peritoneum and connected to a syringe pump (Harvard Apparatus, Holliston, MA, USA). Rats were etherized the awake rats to freely move around. One milliliter isotonic sodium chloride and 2.5 ml ciprofloxacin (ciproxin 2 mg/ml ip; Bayer, Leverkusen, Germany) were administered through the catheter. Buprenorphin (0.3 ml/kg sc) (anorphin 0.3 mg/ml, A/S GEA, Frederiksborg, Denmark) was administered immediately after surgery, ~7 and 20 h post surgery, and when the rats showed signs of pain. Drug control rats were administered a zoletile mixture, ciprofloxacin intraperitoneally, and buprenorphin in similar doses and intervals as rats submitted to surgery.

Automated peritoneal dialysis was initiated within 2 h after surgery and continued for 48 h by using hypertonic Dianeal PD 3.86% (wt/vol) glucose dialysis solution (Baxter, Allerød, Denmark), containing 1,000 IU/l heparin (5,000 IU/ml; LEO; Ballerup, Denmark). At each of the 12 daily exchanges, 10 ml Dianeal was instilled over 10 min and left in the peritoneum for a 30-min dwell time, and then 9.5–9.75 ml (7 ml in sham-operated rats) were tapped during 30 min.

**Blood and dialysate analysis.** Tail arterial blood was obtained from awake rats kept in a constraining cage 2 wk before nephrectomy and/or the autoregulation study and just before the rats were prepared for the autoregulation study. Dialysate for analysis was collected from the outlet-tube from the peritoneum after 48 h of dialysis.

Plasma renin concentration was measured by using a radioimmunochemical microassay by Poulsen and Jørgensen (22). Blood and dialysate samples were analyzed for urea and creatinine by standard chemistry methods at the Department of Clinical Biochemistry. Blood samples were analyzed for hemoglobin (model OSM3; Radiometer Medical, Copenhagen NV, Denmark) and glucose (Yellow Springs Instruments, Yellow Springs, OH).

**Measurement of CBF.** For CBF measurement, the intracarotid 133Xe injection technique adapted for studies in rats was used (9). Anesthesia was induced with 4% halothane in a mixture of 30% O2 and 70% N2O. After tracheostomy, the rats were maintained in a respirator and halothane was reduced to 0.85%. They were paralyzed with pancuronium bromide (pavulon, 2 mg/ml sc (0.4 mg/kg bolus and 0.12 mg/h sc); Organon Teknika, Boxtel, Netherlands). Rats were placed on a heating table and rectal temperature was maintained around 37°C. Femoral veins were cannulated for drug and donor blood administration and femoral arteries were cannulated for continuous blood pressure measurement and blood sampling. Scalp and temporal muscle over the right hemisphere were removed. The right external carotid artery was cannulated and used for the administration of 133Xe solution and extracerebral branches, including the pterygopalatine artery, were ligated to minimize extracerebral distribution of 133Xe. The rats were heparinized (3300 IU/kg iv) and then left to stabilize for 30 min. Arterial Pco2 (Paco2) was kept constant, between 38 and 42 mmHg in lower-limit studies and between 39 and 41 mmHg in time-course studies. Arterial PacO2 (Paco2) was kept above 100 mmHg. For each CBF recording, a bolus of 40–80 μl 133Xe dissolved in saline (3.5 mCi/ml = 370 MBq/ml; DuPont Pharma, Brussels, Belgium) was injected and the clearance of 133Xe from the brain during the first 20 s after the injection was measured. CBF was determined from the initial slope of the washout curve, as introduced in human studies (16) and later developed for use in rats (9). Correction for background and remaining 133Xe activity from previous measurements was performed by recording the activity immediately before each new injection and assuming the remaining 133Xe activity to decay monexponentially during the short period of recording.

After each CBF measurement, mean arterial blood pressures Pao2, Pco2, PacO2, (mmHg), Paco2, (mmHg), Pao2, (mmHg) (catalog nos. ABL 500 and 605; Radiometer, Copenhagen, Denmark), and rectal temperature (°C) were recorded. Baseline recordings (3–4) were made to confirm baseline stability, and these values were averaged for determination of baseline CBF in each rat.

In the time-course studies, CBF recordings were measured at baseline and thereafter at 15-min intervals for 120 min. For the autoregulation studies, blood pressure was reduced stepwise, by controlled bleeding, to the lowest possible level and CBF was measured with ~10-mmHg intervals.

In the group of rats in which the effect of captopril on CBF autoregulation was studied after nephrectomy, baseline CBF measurements were made just after administration of 10 mg/kg iv of the ACE inhibitor captopril (dissolved in isotonic sodium chloride (10 mg/ml); ICN Biomedical, Aurora, OH). This dose of captopril has been used in earlier studies from our group, in which it shifted the limits of autoregulation toward lower blood pressure (2, 3). After the CBF studies, the rats were killed with an overdose of pentobarbital (Nembutal) and examined by autopsy.

**Calculation of the lower limit of CBF autoregulation.** In each autoregulation study, all measurements of CBF of the animal were ranked according to blood pressure values. The lower limit was defined for each rat as the MABP value of the intersection of two lines: a slope line through the points corresponding to the points on the plateau above the limit. To avoid shortcomings leading to meaningless results, constrained calculation methods were used (see APPENDIX for details and Figs. 4 and 5). In the present study, calculation of the lower limit in data from groups (n=8) of rats gave the same results by using plateau and slope constraint, as described in the APPENDIX.

**Statistics.** All data are presented as means ± SD. Blood samples collected before and after intervention were compared by using a paired t-test or the Wilcoxon signed-rank sum test when a normality test failed. The number of paired samples, one-way ANOVA was used with pairwise comparison (Tukey’s test, level of significance P < 0.05). When intergroup comparison failed a normality test or equal variance test, the Kruskal-Wallis one-way ANOVA was used with pairwise comparison (Dunn’s method, level of significance P < 0.05). Intergroup comparision...
son was performed on the difference between blood samples collected before and after intervention.

Baseline values of CBF and blood pressure were compared among the groups by using one-way ANOVA. To investigate the effect of time (time-course studies) within each group, one-way ANOVA was employed, and, when the normality test failed, the Kruskal-Wallis one-way ANOVA rank-sum test was used. CBF and blood pressure changes over time were compared among the four groups by using two-way ANOVA. The limits of autoregulation were compared pairwise after pooling the results from each of the five groups separately and applying an SE estimate of the curves according to a t-statistic testing the intersection of two different lines (13, 38). $P < 0.05$ was considered statistically significant.

RESULTS

Blood analyses and peritoneal clearance. Results of blood analyses and paired t-tests performed within the groups are shown in Table 1. There were no differences between nephrectomy and nephrectomy plus captopril animals. In both groups, plasma renin was below the detection limit 48 h after nephrectomy. A significant fall in plasma renin was also seen in sham nephrectomized animals. The decrease in plasma renin in nephrectomized and sham-operated rats was significantly different from the increase observed in the drug and diet control groups. Large, significant increases in urea and creatinine were found in the nephrectomy groups compared with all other groups. The increase in urea in the drug control group was larger than in sham controls and diet controls. Peritoneal dialysis clearance values of creatinine and urea have been presented in an earlier paper (18).

General condition of the animals. The average food intake and increase in body weight was 26 ± 4 and 8 ± 2 g/day, respectively, when the rats were fed a standard diet and 22 ± 4 and 1 ± 5 g/day when the rats were fed the low-protein, low-potassium diet. After drug administration, food intake almost halted. Post-nephrectomy body weight increased 2 ± 5 g/day, whereas body weight decreased after sham operation (−1 ± 3 g/day) and drug administration (−2 ± 2 g/day, drug-controlled group). Body weights measured just before the CBF recording were 288 ± 26, 278 ± 37, 272 ± 32, 292 ± 27, and 290 ± 25 g in nephrectomized, sham-operated, drug control, diet control, and captopril-treated rats, respectively.

At autopsy, there were no signs of peritonitis in any animal. Five nephrectomized rats and one sham-operated rat were a little edematous at the end of the study due to a partial blockage of the dialysis catheter, resulting in incomplete removal of fluid from the peritoneum. In seven nephrectomized and one sham-operated rat, the catheter had rubbed against the liver and caused small necrotic lesions on the tissue. In five nephrectomized rats, small bruises were found on the stomach or intestine, most likely resulting from the nephrectomy procedure.

Time-course studies of blood pressure and CBF. CBF and blood pressure were stable during the 2-h period of measurement in nephrectomized and control groups. Data from nephrectomy and sham nephrectomy animals are shown in Fig. 1.

Lower limit of CBF autoregulation. Baseline blood pressure was significantly lower in nephrectomized rats compared with all other groups and in sham control rats compared with diet control rats (Table 2). Captopril injection did not change MABP (59 ± 17 mmHg before captopril, 58 ± 10 mmHg after captopril). Baseline CBF was the same in all five groups except for being slightly but significantly higher in sham control rats compared with diet control rats (Table 2).

Lower limits calculated for nephrectomized and sham-operated rats were not significantly different, although they were significantly ($P < 0.001$) lower than in diet control rats (Fig. 2). The lower limit in nephrectomized rats was also significantly lower than that of drug control rats ($P < 0.05$). There were no significant differences in the lower limit between drug and diet controls or between drug and sham controls (Table 2). A further, significant decrease in the lower limit was induced in nephrectomized rats after the administration of captopril ($P < 0.001$) (Table 2, Fig. 2).

Table 1. Blood samples 2 wk before nephrectomy and/or autoregulation study and at the autoregulation study

<table>
<thead>
<tr>
<th>Group</th>
<th>Nephrectomy (n = 31)</th>
<th>Nephrectomy + Captopril (n = 14)</th>
<th>Sham Nephrectomy Control (n = 29)</th>
<th>Drug Control (n = 32)</th>
<th>Diet Control (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renin, BI</td>
<td>0.148 ± 0.125</td>
<td>0.132 ± 0.046</td>
<td>0.133 ± 0.067</td>
<td>0.233 ± 0.197</td>
<td>0.200 ± 0.134</td>
</tr>
<tr>
<td>Renin, AI</td>
<td>BD*</td>
<td>BD*</td>
<td>0.056 ± 0.034*</td>
<td>0.312 ± 0.192</td>
<td>0.406 ± 0.226*</td>
</tr>
<tr>
<td>Urea, BI</td>
<td>6.0 ± 1.1</td>
<td>5.6 ± 0.8</td>
<td>5.5 ± 0.9</td>
<td>5.8 ± 0.9</td>
<td>5.7 ± 0.6</td>
</tr>
<tr>
<td>Urea, AI</td>
<td>35.2 ± 8.6*</td>
<td>39.7 ± 8.1*</td>
<td>4.5 ± 2.5†</td>
<td>7.8 ± 2.7*</td>
<td>4.7 ± 1.4*</td>
</tr>
<tr>
<td>Creatinine, BI</td>
<td>0.038 ± 0.005</td>
<td>0.042 ± 0.003</td>
<td>0.038 ± 0.005</td>
<td>0.042 ± 0.004</td>
<td>0.037 ± 0.004</td>
</tr>
<tr>
<td>Creatinine, AI</td>
<td>0.402 ± 0.068*</td>
<td>0.487 ± 0.061*</td>
<td>0.040 ± 0.007</td>
<td>0.051 ± 0.007*</td>
<td>0.047 ± 0.004*</td>
</tr>
<tr>
<td>Glucose, BI</td>
<td>6.84 ± 0.57</td>
<td>6.90 ± 0.46</td>
<td>6.75 ± 0.44</td>
<td>6.93 ± 0.58</td>
<td>6.97 ± 0.96</td>
</tr>
<tr>
<td>Glucose, AI</td>
<td>5.23 ± 0.87*</td>
<td>5.36 ± 1.18*</td>
<td>6.24 ± 0.63*</td>
<td>6.62 ± 0.76</td>
<td>6.54 ± 0.86*</td>
</tr>
<tr>
<td>Hemoglobin, BI</td>
<td>7.49 ± 0.38</td>
<td>7.62 ± 0.35</td>
<td>7.44 ± 0.43</td>
<td>7.62 ± 0.26</td>
<td>7.47 ± 0.26</td>
</tr>
<tr>
<td>Hemoglobin, AI</td>
<td>7.42 ± 0.77</td>
<td>6.93 ± 0.68</td>
<td>8.33 ± 0.58*</td>
<td>9.29 ± 0.60*</td>
<td>8.82 ± 0.44*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of animals in parentheses used in time course and lower limit of autoregulation studies as well as from blood donor animals. Renin values are in Goldblatt units/l; all other values are in mmol/l. BI, before intervention; AI, after intervention; BD, below detection limit. *$P < 0.001$ and †$P < 0.05$ using paired t-test (for intergroup comparisons, see text).
In the present study, it was demonstrated that the disappearance of circulating renin after bilateral nephrectomy did not influence CBF and its lower limit of autoregulation in rats kept alive by peritoneal dialysis for 48 h. By contrast, in nephrectomized, dialyzed animals, the ACE inhibitor captopril shifted the lower limit of autoregulation toward lower blood pressure. The study also shows the importance of an appropriate method for control groups in such experiments, because a decrease of the lower limit of autoregulation was actually seen in nephrectomized animals but was also seen after sham nephrectomy with dialysis in which circulating renin was present. There was also a tendency for a decrease in the lower limit in the drug control group, which was given analgesics and antibiotics without surgery. Hence, by the present array of control groups, all unspecified effects on CBF autoregulation caused by the experimental setting itself have been accounted for.

In earlier studies in rats, our group has shown that both the lower and upper limits of CBF autoregulation are shifted toward lower blood pressure by acute blockade with captopril or another ACE inhibitor, ceranopril (2, 3, 30, 35). Captopril has to reach the vessel wall

Table 2. Baseline MABP and CBF values collected from time-course studies and lower limit of autoregulation studies

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Lower Limit studies</th>
<th>Baseline MABP, mmHg</th>
<th>Baseline CBF, ml/100 g⁻¹.min⁻¹</th>
<th>Lower Limit, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nephrectomy</td>
<td>8</td>
<td>62 ± 12</td>
<td>106 ± 29</td>
<td>53 ± 4</td>
</tr>
<tr>
<td>Nephrectomy + captopril</td>
<td>8</td>
<td>58 ± 10</td>
<td>94 ± 16</td>
<td>35 ± 2</td>
</tr>
<tr>
<td>Sham nephrectomy control</td>
<td>8</td>
<td>81 ± 9</td>
<td>98 ± 23</td>
<td>58 ± 4</td>
</tr>
<tr>
<td>Drug control</td>
<td>8</td>
<td>93 ± 7</td>
<td>95 ± 16</td>
<td>68 ± 5</td>
</tr>
<tr>
<td>Diet control</td>
<td>8</td>
<td>95 ± 14</td>
<td>111 ± 23</td>
<td>78 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE. MABP, mean arterial blood pressure; CBF, cerebral blood flow.
from the blood side and has no effect on autoregulation when given into the cerebral ventricles (11). In humans, captopril has a similar effect on the lower limit of CBF (36). Autoregulation is mainly a feature of the smaller cerebral resistance vessels, but the converting enzyme inhibitors are probably influencing the blood pressure limits of autoregulation by releasing tone in the larger cerebral resistance vessels (21). The sympathetic α-adrenergic nerves also constrict the larger cerebral resistance vessels rather than the smaller ones (1, 4). In a study of the upper limit of CBF autoregulation in rats, captopril shifted the upper limit markedly toward lower blood pressure, and this shift was attenuated by electric stimulation of the cervical sympathetic chain (35). This indicates some interaction between the renin-angiotensin and the sympathetic nervous system in maintaining the tone of the resistance vessels. By contrast, the decrease in the lower limit of CBF autoregulation is of the same magnitude in normal and sympathetic denervated rats (34).

AT1 receptor blockers in some studies have similar effects as converting enzyme inhibitors on the limits of CBF autoregulation. Thus the AT1 antagonist CV-11947, or candesartan, shifts both the lower and upper limit of CBF autoregulation toward lower blood pressure in spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (32). By contrast, the angiotensin II AT2 antagonist PD-123319 in our laboratory did not influence the lower limit of autoregulation in SHR (5). Other studies of the effect of the AT1 antagonist losartan and PD-123319 have given somewhat contradictory results, and it is possible that these antagonists may display angiotensin receptor agonist activity (15, 25, 26).

It seems most likely that ACE inhibitors and angiotensin II receptor antagonists influence CBF autoregulation by antagonizing angiotensin II-induced vasoconstriction in the cerebral circulation. Other than blocking the conversion of angiotensin I to angiotensin II, the ACE inhibitors also block bradykinin degradation and this might influence the results of the present study. A kallikrein-kinin system is present in the brain, and bradykinin can reach the cerebral blood vessels from both the blood and the brain parenchyma (33). When acting from the perivascular side, bradykinin is a potent dilator of cerebral resistance vessels and has been suggested to be one mediator of cerebral vasogenic edema (33). In a recently published study in Sprague-Dawley rats, it was found that the effect of captopril on the lower limit of CBF autoregulation could be abolished with a bradykinin-2 receptor antagonist, HOE-140 (27). Angiotensin II antagonists, on the

Fig. 3. Schematic presentation of the effects of nephrectomy (elimination of renal renin) and captopril administration (10 mg/kg iv) on blood pressure and CBF autoregulation lower limit. The normal renal function data were from Barry et al. (2, 3). The results in nephrectomized rats are from the present study.

Fig. 4. A–C: stepwise calculation of the autoregulation curve. A: raw data. B: two data points on the slope ($i = 2$); obviously this is not the best fit. C: best fit, lowest sum of squares, obtained for $i = 5$, with a lower limit of CBF autoregulation at 83 mmHg. B and C: filled circles are points on the plateau and open circles are points on the slope.
other hand, which cause a similar effect on the lower limit of CBF autoregulation as do ACE inhibitors (32), do not cause bradykinin accumulation, and hence it is unlikely that such an effect is fully responsible for the effect of captopril in the present and similar studies. As an additional effect, ACE inhibitors but not angiotensin II antagonists may cause bradykinin-mediated nitric oxide accumulation, which has been found in some (12) but not all (37) studies to influence CBF autoregulation, and this could contribute to the present observations of the effect of captopril. Full clarification must await a study of the effect of an angiotensin II antagonist on CBF autoregulation in nephrectomized animals.

The origin of components of the renin-angiotensin system in vessel walls is controversial. Renin and angiotensinogen may be synthesized locally, or may be taken up from the circulating blood (14). In a recent study, angiotensin generation in rat hindquarter vessels had ceased 24 h after bilateral nephrectomy (10). In other studies, after bilateral nephrectomy, plasma renin declined faster than aortic renin and blood pressure responses to converting enzyme inhibition was better correlated with the aortic renin than with plasma renin concentration (28, 29). The inactive precursor of renin, prorenin, also circulates in the blood and may contribute to local renin systems and may, on internalization, be activated in local tissues (23). It has also been proposed that local renin synthesis, e.g., in vessel walls, is suppressed by circulating renin, which almost exclusively originated from the kidneys. Hence, elimination of circulating renin might boost local renin synthesis (19, 20, 31).

The present study affords a dissection of the effects of local cerebrovascular renin-angiotensin as opposed to circulating renin on both blood pressure and the

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**Table 3 Differences between autoregulatory limits calculated using the different modes of calculation**

<table>
<thead>
<tr>
<th>Difference Between Calculated Limits, %</th>
<th>Studies Above the Stated Differences Between Calculated Limits, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1</td>
<td>65</td>
</tr>
<tr>
<td>&gt;5</td>
<td>47</td>
</tr>
<tr>
<td>&gt;10</td>
<td>35</td>
</tr>
<tr>
<td>&gt;15</td>
<td>28</td>
</tr>
<tr>
<td>&gt;20</td>
<td>21</td>
</tr>
<tr>
<td>&gt;25</td>
<td>21</td>
</tr>
<tr>
<td>&gt;50</td>
<td>2</td>
</tr>
<tr>
<td>&gt;100</td>
<td>2</td>
</tr>
<tr>
<td>Identical limits seen in</td>
<td>35</td>
</tr>
</tbody>
</table>

Const., constraint.
lower limit of CBF autoregulation. This is illustrated in Fig. 3. In an earlier study (2), we found that intravenous captopril lowered blood pressure and the lower limit of autoregulation to the same percentwise extent. In the present study, we found that nephrectomy lowered blood pressure to the same extent as captopril in the earlier study, but did not influence the lower limit. Captopril given to nephrectomized animals did not, by itself, decrease blood pressure further but markedly lowered the lower limit of autoregulation. Thus in our hands, the effect of captopril on blood pressure appeared to depend on circulating, renally derived renin and angiotensin, whereas the effect on CBF autoregulation depended on local renin situated in the vessel wall. A contribution of bradykinin accumulation on either effect cannot be ruled out.

In conclusion, the effect of captopril on the lower limit of CBF autoregulation was still present 48 h after bilateral nephrectomy when circulating renin could no longer be detected. Captopril thus exerted a local intravascular effect, with a possible contribution of bradykinin accumulation.

**APPENDIX**

**Modes of Calculation of the Lower Limit of Autoregulation**

The autoregulatory curve can be determined by repeated measurement of CBF and MABP. But, the exact blood pressure value at the limits of autoregulation is not easily defined. The lower limit could be calculated as the intersection point of a simple slope line including points below the lower limit and a simple horizontal plateau line including the points above this limit. However, this simple calculation has many shortcomings for which reasons constraints are introduced.

In each study, all measurements are ranked according to blood pressure values (from 1 to N).

**Simple calculation—no constraint.** The lower limit of autoregulation is calculated as the breaking point between a slope regression line through the points below the lower limit, and a horizontal regression line corresponding to the points on the plateau, above the limit (Fig. 4, A–C). The slope can include points ranging from i = 2 to N – 1, and the remaining points (N – i) represent the plateau. When calculating the lower limit, more and more points are included in the slope (Fig. 4B) until the best fit is obtained, defined as the least sum of squares of the deviations from the different sets of lines (Fig. 4C).

However, using this approach on real data revealed many shortcomings, and this calculation model turned out to be insufficient. Thus, as data obtained with this mode of calculation can lead to meaningless results (Fig. 5B), two constrained calculation models are used: plateau constraint and slope constraint.

**Plateau constraint.** Plateau constraint was introduced by Schmidt et al. (24) (Fig. 5). In this model, as in the unconstrained model, more and more points (i) are included on the slope and the best fit of the two intersecting lines, with the lowest sum of square calculated. However, a constraint is put on the horizontal line, which is confined to cross the slope between the blood pressure values (i) and (i + 1). When the average horizontal line would be above these blood pressure values, then a line corresponding to the blood pressure value (i + 1) is used, and when the horizontal line would be below the blood pressure values, a line corresponding to i is used (Fig. 5C). Thus, in this model, the points on the slope defines its inclination, and then the plateau level is set (constrained) between the last point on the slope and the first point on the plateau.

**Slope constraint.** Calculation of the autoregulatory limits with a slope constrain was for the first time introduced in the present communication (Fig. 5). If the slope crosses the horizontal line outside the blood pressure interval between point i and i + 1, then a new slope line is constructed going through the point i or i + 1, depending on whether the slope crosses below or above the interval (Fig. 5D). The slope is then calculated by the least-sum-of-square method. Thus, in this model, the points on the plateau define its level. The slope intersection with the plateau is then set (constrained) between the lowest point on the plateau and the highest point on the slope, and the inclination of the slope is then calculated by the least-sum-of-square method.

**Statistics**

With the calculation of the autoregulatory limits as described above, it is possible to calculate the SE of the estimate of the curve according to a t-statistic testing the insertion from two different lines (13, 38).

**Evaluation**

After going through data from ~40 autoregulation measurements, it becomes apparent that calculation of the lower limit by using no constraint may give conflicting nonphysiological results. It is astonishing that calculation using the two different constrained approaches gives essentially the same results with only minor deviations in a few cases (Table 3). This illustrates the robustness of the slope and plateau regression line calculations using either of the constraining methods. The SE of the estimate of the autoregulatory curves averaged in these 40 data sets is 4.5 ± 3.0 mmHg (7.5 ± 3% of the lower limit value).

We thank Inge Møller for meticulous laboratory work during the study.

**DISCLOSURES**

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