No effect of angiotensin II AT₂-receptor antagonist PD 123319 on cerebral blood flow autoregulation

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Abstract
Blockade of the renin-angiotensin system with angiotensin-converting enzyme inhibitors (ACE-I) or angiotensin AT₁-receptor antagonists shift the limits of autoregulation of cerebral blood flow (CBF) towards lower blood pressure (BP). The role of AT₂-receptors in the regulation of the cerebral circulation is uncertain. Hence, the present study investigated the effect on CBF autoregulation of blocking of angiotensin AT₂-receptors with PD 123319 intravenously, 0.36 mg/kg/min, and compared with a control group. PD 123319 was raised by noradrenaline infusion and lowered by controlled haemorrhage in separate groups of rats. The limits of autoregulation were determined by computed least-sum-of-squares analysis. PD 123319 did not influence baseline CBF, but was found in a significant BP decrease (10 control and 10 treated rats). The lower limit of CBF autoregulation (eight treated and eight control) was found along with the upper limit of CBF autoregulation (eight treated and eight control) was not significantly different in PD 123319 and control animals (lower limit treated 102±4 mmHg and control 94±4; NS, and upper limit treated 171±10 mmHg and control 162±7; NS). These findings indicate that the AT₁-receptor blockade does not influence CBF autoregulation.

Introduction
Cerebral blood flow (CBF) is autoregulated, i.e. is kept constant within a wide range of perfusion pressure. Autoregulation of CBF is mediated mainly by changes in the smaller cerebral resistance vessels, which consist when perfusion pressure increases, and dilate when perfusion pressure decreases. Beyond the upper limit of autoregulation, the arteries and arterioles are unable to constrict further, and the CBF rises, eventually resulting in blood-brain barrier, cerebral oedema formation and risk of cerebral haemorrhage. Below the lower limit of CBF autoregulation, the arteries and arterioles will be submaximally dilated and as the perfusion pressure falls, CBF will fall, resulting in cerebral ischaemia, with risk of irreversible damage.

The renin-angiotensin system (RAS) modulates CBF autoregulation. Angiotensin-converting enzyme inhibitors (ACE-I) and AT₁-receptor blockers (ARBs) shift both the upper and lower limits of autoregulation towards lower blood pressure (BP) and shorten the autoregulatory plateau. It has been suggested that selective blockade of the AT₁-receptor in large cerebral arteries causes dilation of these large cerebral arteries with a compensatory autoregulatory constriction further downstream in small arteries and arterioles.

The function of the AT₂-receptor is poorly understood. It is less widely distributed in the body than the AT₁-receptor. The pressor effect of angiotensin II (Ang II) is mediated by the AT₁-receptor, and the role of the AT₂-receptor in BP regulation is still unclear. All of the well-known effects of Ang II, such as aldosterone, vasopressin and oxytocin release, negative feedback on renin release, and renal salt and water retention are mediated by the AT₁-receptor. Previous studies have failed to clarify the function of the AT₂-receptor in the cerebral circulation.

The aim of the present work was to investigate whether blockade of this receptor influences autoregulation of CBF. The study was undertaken in spontaneously hypertensive rats (SHR) using the selective AT₂ antagonist, PD 123319.

Methods
The study was carried out in 52 male SHR, aged around three months and weighing between 200 and 350 g. The rats were supplied by Møllegården, Lille Skensved, Denmark. They had unlimited access to food and water prior to the study.

Surgery
Anaesthesia was induced with 4% halothane in a mixture of 30% O₂ and 70% N₂O. After tracheotomy, anaesthesia was maintained with 0.8% halothane in 30% O₂ and 70% N₂O, by controlled ventilation at normocapnia. Rats were paralysed with suxamethonium i.p. (10 mg/kg bolus and 3.5 mg/kg/hour), in order to prevent interference from spontaneous respiration with regard to the stability of the arterial carbon dioxide tension (PaCO₂). A rectal thermostat-controlled heating table maintained body temperature at around 37°C. Both femoral veins were cannulated (pp50 catheter, Holm & Halby, Denmark) for drug and donor blood administration. Both femoral arteries were cannulated (pp50 catheter, Holm & Halby, Denmark), one for continuous BP measurement with a transducer (Simonsen & Weel, Denmark), and one for blood gas sampling. PaCO₂, PaO₂ and pH were measured, using an arterial blood gas
analysers (ABL 500 and 605, Radiometer, Denmark). PaCO₂ was kept constant (between 37 and 41 mmHg) during the experiment by adjusting the ventilation volume. For CBF measurement, the intravascular 133Xe technique was used. The scalp and the temporal muscle over the right hemisphere were removed. The right external carotid artery was exposed on the right side near the carotid bifurcation. Extracerebral branches, including the pterygopalatine artery, were ligated, in order to minimise extracerebral distribution of 133Xe. Rats were then heparinised (3500 IU/kg), and a fine catheter (pp25, Holm & Halby, Denmark) was introduced retrogradely into the external carotid artery, with the tip at the bifurcation, for 133Xe administration. After surgery, anaesthesia was maintained with 0.6% halothane in 30% O₂ and 70% N₂O, and the rat was left to stabilise for 30 minutes, before any CBF measurement was made.

Cerebral blood flow measurement
For each CBF measurement a bolus of 30–70 µl 133Xe, 3.5 mCi/ml (370 MBq/ml) was injected into the right internal carotid artery. A collimated sodium iodide crystal placed over the skull ipsilateral to the injection site, measured the clearance of 133Xe from the brain during the first 20 seconds after the injection. CBF was then determined from the initial slope of the washout curve. The correction for background activity and any activity remaining from previous 133Xe injections was slightly different, as we recorded the data directly on a computer allowing for a mathematical correction. The correction was the background recorded before any 133Xe plus the remaining 133Xe recorded immediately before the new injection and assumed to decay with a constant time constant during the short period of recording. At the end of each measurement MABP (mmHg), CBF (ml/100 g/min), PaCO₂ (mmHg), PaO₂ (mmHg), arterial pH and rectal temperature (°C) were recorded. Three baseline measurements were made before drug administration and manipulation of the BP, to confirm baseline stability. The values were averaged for the determination of baseline CBF level in each rat.

PD 123319 administration
PD 123319 (Parke-Davis, Michigan, USA) dissolved in saline, 0.36 mg/kg/min, was administered by intravenous infusion (0.56 ml/hour). PD 123319, at doses of 0.36 and 1 mg/kg/min, has been shown not to affect baseline CBF. The lowest dose was chosen in order not to interfere with AT₁-receptors.

Time course of the effect of PD 123319
PD 123319 was administered to 10 SHR. Ten rats received vehicle (saline) as control. CBF was measured at baseline and at 2, 5 and 10 minutes during drug administration. Thereafter, CBF was measured at 15 minute intervals for 120 minutes.

Auto regulation study
The lower limit of CBF autoregulation was studied in 16 SHR. Eight animals received PD 123319, while eight served as controls. PD 123319 or saline was administered intravenously, and the BP was allowed to stabilise for 10 minutes after the injection and prior to the commencement of the autoregulation study. Haemorrhagic hypotension was subsequently induced by withdrawing blood into a syringe. By this means, BP was reduced stepwise to the lowest obtainable level. Throughout the study, CBF was measured at 10 to 15 mmHg BP intervals.

The upper limit of CBF autoregulation was studied in 16 SHR. Eighte received PD 123319 and eight served as controls. BP was increased gradually to the highest obtainable level by the intravenous infusion of norepinephrine (NE), 0.025–0.5 µg/min, and CBF was measured at 10 to 15 mmHg BP intervals.

Investigation of the lower and upper limits of CBF autoregulation were made in separate groups of rats, since rats are no longer in a normal physiological condition when their BP has been brought to the extremes outside the limits of autoregulation.

Calculation of the autoregulation curve
The lower and upper limits of CBF autoregulation in each individual rat were defined as the mean arterial blood pressure (MABP) value of the intersection of two lines: one slope regression line for all points from 1 to i, and one horizontal line for the point i + 1 to N (all measurements ranked in order of increasing BP). The horizontal line had to cross the slope line between the slope MABP values at i and i + 1, but was otherwise chosen as, or as close as possible to, the mean of the points i + 1 to N. Going through all data points (i = 2 to N - 2), the best fit for the autoregulation curve was then defined by the least-sum-of-squares method of the combined line. Autoregulation was defined as preserved when the total sum of squares for the best fit was smaller than that of one straight regression line through all data points. The upper limit of autoregulation was calculated using the horizontal line corresponding to the data point with lower BP values and the slope corresponding to those with higher BP's. Otherwise, calculations were performed as for the lower limit.

Data analysis
The Wilcoxon rank sum test was used for intergroup comparisons of the baseline values from the time-course study and the autoregulation study. Analysis of variance (ANOVA) was used for statistical comparison of the time-course groups (control versus PD 123319). The Wilcoxon rank sum test was used for statistical comparison of the effect of time in the time-course group. Values are expressed as mean±SD. The limits of autoregulation were evaluated by pooling all control and PD 123319 animals separately and applying a SE estimate of the curves, according to a t-statistics test, testing the intersection of two different lines. Values are expressed as mean±SEM. p<0.05 was considered statistically significant.

Results
Baseline values in the different groups are shown in
Table 1. There were no significant differences in any of the baseline parameters between the groups (p>0.05), except for pH (p<0.01) and temperature (p<0.05) in the time-course groups and pH (p<0.05) in the lower limit groups. These alterations were very small, and not likely to influence the results of the study. PaCO₂, PaO₂, pH and temperature were constant and within the normal range during the study, except for slight alterations caused by extreme hypotension and hypertension induced at the end of the experiment.

Time-course study

Effect of PD 123319 on mean arterial blood pressure

Administration of PD 123319, 0.36 mg/kg/min, caused a significant decrease in MABP in the time-course study. During the first 10 minutes, there was no reduction in MABP, (138±21 to 141±23 mmHg, p>0.05). During the rest of the study, there was a significant reduction in MABP (143±12 to 112±13 mmHg, p<0.001). The overall reduction was from 138±21 to 112±13 mmHg (p<0.01). The BP-lowering effect commenced approximately one hour after the start of the drug infusion (Figure 1A). The difference in BP between the control and PD 123319-treated groups was significant only for the last measurement (time = 120 minutes, Figure 1A).

Effect of PD 123319 on cerebral blood flow

There was no effect on CBF during the time-course study, (92±11 to 94±11 ml/100 g/min, p>0.05). (Figure 1B)

Lower limit of cerebral blood flow autoregulation

CBF autoregulation was preserved in both PD 123319-treated and control groups. The lower limit of CBF autoregulation was 94±3.8 in the control group and 102±3.9 mmHg in the PD 123319-treated group (NS).

Upper limit of cerebral blood flow autoregulation

CBF autoregulation was preserved in both PD 123319-treated and control groups. The upper limit of CBF autoregulation was 162±7.1 in the control group and 171±10.2 mmHg in the PD 123319-treated group (NS).
In Figure 2, normalised CBF values from the studies of the lower and upper limits have been combined to show the whole autoregulation curve in control (2A) and PD 123319-treated (2B) animals.

Comparison with earlier studies from our group
In Table 2, results from studies made by our group with the ACE-I, captopril, and ceranapril, and the ARB, candesartan, are shown. ACE inhibition and AT1-receptor blockade, in contrast to AT2-receptor blockade, shifts the limits of CBF autoregulation to lower pressure limits.

Discussion
The main observation of the present study was that PD 123319 had no effect on the limits of autoregulation. Furthermore, PD 123319 significantly lowered MABP in the time-course study, without influencing CBF.

It is unlikely that the methodology of the study would have obscured any effect of PD 123319 on CBF autoregulation. The intra-arterial 133Xe injection method for CBF measurement in the rat, as used in this study, allows repetitive measurements at short intervals, and is thus well suited to the study of cerebrovascular reactivity. The method requires universal anaesthesia, which might influence CBF. In the present study, the rats were anaesthetised with halothane and paralysed with suxamethonium. Both of these agents have ganglion-blocking properties, but any effect on CBF would be present in both control and in AT1-receptor antagonist studies, and thus are unlikely to influence the comparisons between the groups. Anaesthesia with halothane causes a decrease in MABP, without an increase in plasma renin activity, but again any effect on CBF would be present in both groups. Norepinephrine has no effect on CBF. Haemorrhagic hypotension leads to α-adrenergic vasoconstriction, which shifts the autoregulatory curve towards higher BP. This effect, blunted by halothane and suxamethonium, would be present in both control and PD 123319-treated groups.

Ang II receptors in large cerebral arteries in rats have been suggested to be of the AT2-receptor subtype. However, the AT1-receptor subtype is also expressed, at least in the middle cerebral artery. Ang II-mediated vasodilation has been found in rat brain arterioles and in dog middle cerebral arteries. Ang II-mediated vasoconstriction has been reported in cat middle cerebral artery strips. This dual effect of Ang II suggests stimulation of two types of Ang II receptors in the...
cerebral circulation, with AT1-receptors mediating vasoconstriction and AT2-receptors mediating vasodilation. ACE-I shift the limits of CBF autoregulation towards lower BP. In a previous study from our group, the ARB CV-11974 (candesartan) was shown to have a similar effect, whereas the present study shows that AT2-receptor blockade has no effect on CBF autoregulation (Table 2). Studies by other groups have given somewhat contradictory results. Thus, Strömberg and co-workers showed that PD 123319 and losartan, an ARB, both shifted the upper limit of CBF autoregulation towards higher BP. They suggested that stimulation of the AT2-receptor causes vasoconstriction, and that vasodilation is mediated through the AT1-receptor. According to these studies, PD 123319 functions as an agonist at the AT2-receptor, causing vasoconstriction, and losartan acts as an antagonist at the AT1-receptor; also causing vasoconstriction, resulting in a shift of the upper limit of autoregulation towards higher BP. In a previous report, Strömberg and co-workers found that losartan shifted the upper limit of CBF autoregulation towards lower BP, which is consistent with a vasoconstrictory role of the AT2-receptor. These earlier results of Strömberg and co-workers are in agreement with Vraamark and co-workers. Another study in rats revealed that blockade of AT1-receptors, in contrast to blockade of AT2-receptors, abolished the blood flow reduction resulting from intracarotid Ang II infusion. The BP-lowering effect of PD 123319, as demonstrated in the time-course study, is difficult to explain. Although the PD 123319 is not absolutely specific for AT2-receptors, it is unlikely that it had any major effect on AT1-receptors.

The absence of a significant cerebrovascular response to PD 123319, as observed in the present study, could be due to the nature of the AT2-receptor in the SHR strain. Endo and co-workers recently showed that Ang II caused more marked vasoconstriction in SHR kidney afferent arterioles than in WKY afferent arterioles, possibly due to an impaired modulator function of the AT2-receptor in SHR afferent arterioles. SHR was chosen in the present study because it is a model of human essential hypertension. Carried out in WKY rats, a more pronounced effect on CBF autoregulation might have been seen with a shift of the limits of autoregulation towards even higher BP. In conclusion, the present study showed no significant effect of the AT2-receptor on CBF autoregulation in SHR.

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