Enhanced reactivity towards flunarizine in cerebrovascular bed of spontaneously hypertensive rats

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Summary. Cerebral blood flow (CBF) was measured and cerebrovascular resistance (CVR) was calculated in anesthetized spontaneously hypertensive rats (SHR) and normotensive rats (NR) following the administration of incremental dosages of i.v. flunarizine or papaverine. CBF and CVR changes following papaverine were the same in both groups of rats irrespective of the dose of the drug. The effect of flunarizine was much more pronounced in SHR than in NR. The results point out the greater dependency of basal cerebrovascular tone in SHR upon Ca^{2+} influx into vascular smooth muscle cells.
Key words. Cerebral blood flow; cerebrovascular resistance; SHR; NR; flunarizine; papaverine.

Elevated CVR in SHR is considered to be a result of arteriolar wall hypertrophy[1, 2] which results in greater CVR in SHR also during maximum vasodilation[3]. The coexisting enhanced sympathetic drive can also add a component to exaggerated CVR[4]. As the next constituent of elevated CVR one should consider the possibility of defective permeability of the cellular membranes and thus, independent of sympathetic activation, a greater influx of calcium ions into the smooth muscle cells in SHR[5]. Recent results suggest increased dependence of the vessel tone upon calcium ions influx in the peripheral vascular bed in essential hypertension in humans and SHR[6]. The present study was undertaken to find out whether this is true also for the cerebrovascular bed in SHR. To block Ca^{2+} entry into smooth muscle cells, flunarizine was chosen. This drug belongs to the diphenylalkylamine group of Ca^{2+} antagonists and does not affect myocardium[7] and spontaneous myogenic activity in vascular smooth muscle[8, 9]. The effect of flunarizine was compared with papaverine, which instead of binding to the cell membrane influences mainly intracellular Ca^{2+} sequestration and can affect calcium influx secondary to the inhibition of phosphodiesterase[10, 11].

Materials and methods. The study was carried out on 25 3–4-month-old male SHR and 24 age-matched NR (Wistar) anesthetized with Nembutal (35 mg/kg i.p.) and paralyzed with gallamine (0.5 mg/kg i.v.) in order to control ventilation. The min-volume of the respiration was adjusted as necessary in order to maintain normocapnia (P_aCO_2 33–40 mm Hg). The animals were ventilated with 30% O_2 in air. PO_2 was closed to 100 mm Hg. Body temperature was controlled and maintained around 37 °C with a heating pad. Catheters were introduced into the abdominal aorta and the inferior vena cava through the femoral vessels to allow measurements of mean arterial blood pressure (MAP) and the infusions, respectively.

CBF was determined by the intracarotid injection of the radioactive inert gas 133-Xe according to the method introduced by Hertz et al.[12]. The catheter for 133-Xe administration was placed centripetally in the external carotid artery after ligation of the pterygopalatine and of small branches of the external carotid artery on the side of the isotope injection. For the CBF measurements, a bolus of 133-Xe was injected in a volume of 15–30 µl physiological saline solution. The radioactivity of xenon was detected by a well-collimated scintillation crystal attached to a photomultiplier mounted over the ipsilateral temporoparietal region. CBF was calculated from the initial slope (15 s) of the logarithmically displayed clearance curve and expressed in ml/100 g/min. The mean coefficient of variation for repeated measurements of CBF was 6% in a control state. CBF measurements in the control state (base line values) were made in duplicate and the values averaged.

CVR was calculated as MAP devided by CBF and was expressed as mm Hg/ml/100 g/min. Blood gas analysis was performed before each CBF measurement. CBF was determined and CVR calculated in NR and SHR before and 3, 15 and 30 min after administration of flunarizine (Janssen Pharmaceutica) or papaverine (Merck Lab.). Flunarizine was dissolved in distilled water to which Tween II was added in order to facilitate solution. Both drugs were given in increasing doses (0.1, 0.5, 1.0 mg/kg) as a 0.5-ml intravenous bolus. In the pilot series with flunarizine control CBF was measured 3 min after i.v. administration of vehicle (0.5 ml) in order to exclude the effect of the solvent on CBF. In each rat, only one drug was studied. Because flunarizine is metabolized slowly, only one dose of this drug was studied in each rat. The statistical analysis was done by means of Student's t-test; a probability value of less than 5% was considered significant ($p < 0.05$).

Mean arterial blood pressure, cerebral blood flow and cerebrovascular resistance in NR and SHR under basal conditions. The values are given as mean ± SEM, NS denotes no significant difference

Subject	MAP (mm Hg)	CBF (ml/100 g/min)	CVR (mm Hg/ml/100 g/min)
NR	118 ± 7.9	78.9 ± 5.6	1.48 ± 0.08
SHR	203.0 ± 7.5	76.2 ± 3.2	2.39 ± 0.11
p	< 0.001	NS	< 0.001

Results. Control CBF does not differ between SHR and NR (table). CVR is 61% greater in SHR than in NR. This difference is diminished during vasodilation (fig. 2), but CVR in SHR is still significantly greater than in NR ($p < 0.001$). Cerebral vasodilation due to both compounds is short-lasting. The changes in CBF are observed only at 3 min following an i.v. bolus of either papaverine or flunarizine. The dose-dependent effects of the drugs on CBF and their effect on CVR are summarized in figures 1 and 2 respectively. The papaverine-elicited increase in CBF and decrease in CVR are

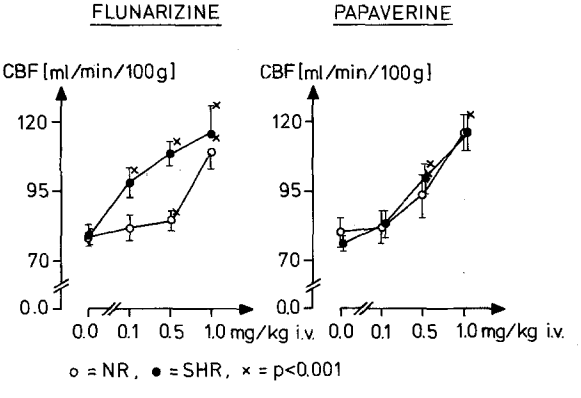

Figure 1. Changes in CBF in response to incremental dosages of i.v. administered flunarizine and papaverine in 6 NR (open circles) and 7 SHR (filled circles). Values are given as mean ± SEM.

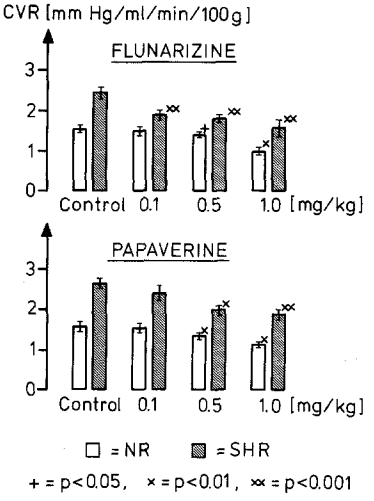

Figure 2. CVR following incremental dosages of i.v. administrated flunarizine and papaverine in 6 NR (open bars) and 7 SHR (hatched bars). Values are given as mean ± SEM.

the same in NR and SHR. The threshold dose results in an increase in CBF of about 17% in NR (from 78.9 ± 5.6 to 92.6 ± 8.9 ml/100 g/min) and 28% in SHR (from 76.2 ± 3.2 to 98.0 ± 6.8 ml/100 g/min). 1.0 mg/kg papaverine results in an increase in CBF of 33.5% in NR and 44.0% in SHR; the difference between the two groups of rats is again not significant. CVR was diminished following a threshold dose of the drug, by 8% in NR (from 1.44 ± 0.10 to 1.33 ± 0.12 mm Hg/ml/100 g/min) and 15% in SHR (from 2.35 ± 0.12 to 2.0 ± 0.13 mm Hg/ml/100 g/min). Attenuation of CVR following 1.0 mg/kg papaverine amounted to 20% of the control value in both groups of rats.

The effect of flunarizine is unequivocally more pronounced in SHR than in NR. The theshold dose of flunarizine in SHR (0.1 mg/kg) results in an increase in CBF of 29% (from 76.3 ± 3.2 to 98.9 ± 7.9 ml/100 g/min) and a decrease in CVR of 22% (from 2.43 ± 0.16 to 1.9 ± 0.16 mm Hg/ml/100 g/min). This dose has no effect on either parameter in NR. The highest dose of this compound has the same effect on CBF and CVR in both groups of rats.

The MAP decrease following papaverine or flunarizine lasted no longer than 60 s. In SHR the threshold dose, 0.1 mg/kg of papaverine, diminished MAP by 12% and was without effect on MAP in NR. The changes in MAP in NR were seen only following 1.0 mg/kg papaverine and were of the same order of magnitude as in SHR (about 20% of the control value). Following 0.5 and 1.0 mg/kg flunarizine, MAP decreased in SHR by 17% and 20%, respectively. MAP in NR was affected only by 1.0 mg/kg flunarizine. The decrease in MAP after this dose was the same as in SHR, i.e., 21%.

Discussion. A brief summary of these results points to a greater dependence of the basal cerebrovascular tone upon Ca^{2+} entry into vascular smooth muscle in SHR than in NR. The threshold for CBF and CVR responses to flunarizine was lower in SHR than in NR. Only one out of three doses of flunarizine affected CBF and CVR in SHR and NR to the same degree. The cerebrovascular reactivity towards papaverine was the same in both groups of rats irrespective of the dosage of the drug administered. Although both drugs studied interfere with the influx of Ca^{2+} through the cell membrane, their mechanism of action appears to be different. Flunarizine, although not selective, blocks the calcium channel by binding to plasma membrane sites [13]. Flunarizine does not block spontaneous myogenic activity in vascular smooth muscle but inhibits Ca^{2+}-influx when it is exaggerated (Ca^{2+}-overload) [14]. It is known from in vitro studies that diphenylalkylamines can also interact directly with contractile proteins in smooth muscle when administered in high doses [15]. A similar phenomenon could be responsible for the lack of differences between SHR and NR in the effect on CBF and CVR of the highest flunarizine dose (1 mg/kg). Papaverine, in contrast to Ca^{2+} antagonists, does not specifically inhibit contractions dependent on extracellular calcium ions [16]. It affects mainly intracellular Ca^{2+} binding, enhancing Ca^{2+} sequestration due to inhibition of phosphodiesterase. Its influence on Ca^{2+} membrane permeability could be secondary to intracellular action, which should cause facilitation of calcium influx rather than inhibition [17]. Present results would point to calcium overload in cerebrovascular smooth muscle in hypertension. It could be due to enhanced sympathetic stimulation or to a primary defect in calcium handling in essential hypertension [4, 5, 18, 19]. Thus, apart from reported structural changes in cerebral resistant vessels in essential hypertension [1, 3], functional abnormalities also participate in the enhancement of cerebrovascular resistance in SHR. On the basis of the present results it seems that these functional changes, at least as far as calcium ions are concerned, consist of an exaggerated influx of ions through the cell membranes.

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