Hypertension and brain oedema: an experimental study on acute and chronic hypertension in the rat

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SUMMARY To determine under what circumstances hypertension is associated with brain oedema, the specific gravity of the brain was measured in acutely hypertensive, renal hypertensive and spontaneously hypertensive rats. Maximum mean arterial pressure (MAP) in acute hypertension induced by intravenous amphetamine or bicuculline was 171±5 and 181±5 mmHg respectively. In spite of pronounced extravasation of Evans blue-albumin, there was no decrease in specific gravity except in the diencephalon in rats given bicuculline (p<0.05). Cortical and cerebellar samples from renal hypertensive rats (MAP 174±14 mmHg) were lighter than corresponding regions in normotensive rats (p<0.001) although the brains showed little or no macroscopic extravasation of Evans blue-albumin. Neither macroscopic protein leakage nor increase in water content was observed in brains from spontaneously hypertensive rats (MAP 210±5 mmHg). It is concluded that renal hypertension is more likely to lead to brain oedema than spontaneous genetic hypertension or acute hypertension.

Although it is known from clinical experience as well as from animal studies that there is a dissociation between albumin leakage and oedema in ischaemic cerebral lesion, extravasation of protein in the brain is often referred to as brain oedema. It is frequently assumed that the permeability increase per se leads to brain oedema, that is an increase in water content of the "vasogenic" type. Hypertension greatly enhances the tendency for oedema in the presence of traumatic brain lesions, but it is not clear to what extent a blood-brain barrier (BBB) dysfunction in the absence of tissue damage leads to brain oedema even at high levels of perfusion pressure. Studies on acute hypertension with disturbed BBB function have so far shown no increase in water content unless hypertension was combined with severe hypercapnia. Likewise, little or no brain oedema has been observed in experimental seizures, a condition with an abrupt increase in blood pressure and pronounced cerebral vasodilatation. It has been suggested that the failure to demonstrate brain oedema in acute hypertension might be due to the limited precision of the conventional methods of weighing and drying tissue samples for detecting brain oedema. Changes in brain water content can be derived from specific gravity measurements provided that the specific gravity of tissue solids remains constant. A sensitive technique to measure changes in brain water content based on determination of the specific gravity in a gradient column has been developed by Nelson et al. They reported that the range of specific gravity determined by their method was 10--20 times less than that found by weighing whereas the two methods gave the same mean specific gravity value. Several later studies have confirmed the high precision of the gravimetric method in detecting brain oedema. In the present study we used this technique to determine specific gravity of brain tissue in acutely and chronically hypertensive rats. Since vasodilatation enhances protein leakage in the brain during acute hypertension, two drugs which increase the blood pressure and in addition give rise to cerebral vasodilatation were used, that is bicuculline, a drug that provokes seizures and amphetamine. Renal hypertensive and spontaneously hypertensive rats were studied as models of chronic hypertension.
Materials and methods

Male Sprague-Dawley rats (200–300 g) were used except in two groups when the stroke-prone substrain of the Japanese spontaneously hypertensive rats (SHRSP) and Kyoto Wistar control rats (KWR) were investigated. Only normocapnic animals with PaO₂ > 11 kPa were studied. Evans blue, which in vivo binds to serum albumin, was given intravenously (2 ml/kg of a 2% solution) at the start of the experiments. For the number of rats in each group, see the table.

Controls Rats were decapitated under diethyl ether, methohexital or nitrous oxide anaesthesia under conditions comparable to those in the experimental groups, that is they were kept anaesthetised for the same period of time and received the same amount of fluid as the experimental animals. Three of the rats anaesthetised with diethyl ether had indwelling catheters as described for renal hypertensive and spontaneously hypertensive rats.

Amphetamine Anaesthesia was initiated with methohexital (Brietal® 50 mg/kg). One femoral artery and one femoral vein were cannulated for anaerobic sampling of blood for blood gas determination, for continuous recording of MAP and for injection of drugs and tracers. The rats were then tracheotomised, relaxed with suxamethonium chloride and ventilated with 75% N₂O in O₂ in a rodent respirator (Harvard apparatus). d, l-amphetamine (5 mg/kg), was given intravenously and the rats were killed by decapitation after an additional dose of methohexital 30 minutes later.

Bicuculline was injected intravenously (12 mg/kg). The operative procedure was the same as described for amphetamine. Decapitation was performed when MAP fell below 130–140 mmHg, which occurred within 20 minutes after the bicuculline administration in six rats and after 80 and 200 minutes in two rats.

Renal hypertension The left renal artery was constricted to 0·20 mm by a silver clip under methohexital anaesthesia in rats weighing approximately 175 g. Eight weeks later indwelling catheters were inserted in the aorta from the left femoral artery and in the jugular vein and exteriorised on the back of the neck under methohexital anaesthesia. Two days later the blood pressure was recorded in conscious, unrestrained rats for one hour. If MAP exceeded 160 mmHg, Evans blue was injected intravenously and the rats decapitated after intravenous injection of methohexital 2–3 hours later.

Spontaneously hypertensive rats Indwelling catheters were inserted 2 days before killing in SHRSP and control WKR and the rats (10–12 months of age) were treated in the same way as described for renal hypertensive rats.

After decapitation the brains were rapidly removed and immediately immersed in cool kerosene. The specific gravity was determined in a gradient tube made of 250 ml of two different bromobenzene-kerosene mixtures of specific gravity 1·0650 and 1·0350 respectively, the former being continuously diluted by admixture of the latter while poured into a graded glass tube. This was placed in a wider glass tube containing water. Stability of linearity was controlled by standard solutions of K₂SO₄ in connection with determination of every sample series. Samples were taken from the cortex, mesencephalon, diencephalon, pons and the cerebellum. To clear the gradient tube, dry Na₂SO₄ was used. Statistical differences were evaluated with Wilcoxon's rank sum test.

Results

Initial MAP and maximum MAP after injection of amphetamine were 135±7 and 171±5 mmHg (mean±SEM, see table for the numbers). The corresponding values for rats given bicuculline were 121±9 and 181±5 mmHg. MAP in anaesthetised controls was 132±5 mmHg. In conscious, unrestrained renal hypertensive rats MAP was 174±14 mmHg, in SHRSP 210±5 mmHg, in WKR 112±4 mmHg and in Sprague-Dawley controls 108±5 mmHg. All brains from rats given amphetamine or bicuculline showed areas of Evans blue-albumin extravasation in contrast to

| Table Specific gravity of the brain in hypertensive rats compared to control rats |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Experimental group | n | Frontal cortex | Parietal cortex | Diencephalon | Mesencephalon | Pons | Cerebellum |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Control (S-D) | 12 | 1·0452±0·0001 | 1·0452±0·0002 | 1·0437±0·0001 | 1·0431±0·0002 | 1·0423±0·0001 | 1·0464±0·0003 |
| Control (WKR) | 6 | 1·0452±0·0001 | 1·0452±0·0002 | 1·0437±0·0001 | 1·0430±0·0001 | 1·0419±0·0001 | 1·0464±0·0003 |
| Acute hypertension | 8 | 1·0449±0·0001 | 1·0449±0·0002 | 1·0428±0·0004 | 1·0427±0·0002 | 1·0421±0·0002 | 1·0461±0·0003 |
| d, l-Amphetamine (5 mg/kg) | 8 | 1·0450±0·0002 | 1·0450±0·0003 | 1·0431±0·0002* | 1·0429±0·0002 | 1·0422±0·0003 | 1·0450±0·0010 |
| Bicuculline (12 mg/kg) | 8 | 1·0435±0·0002 | 1·0436±0·0002 | 1·0432±0·0002 | 1·0428±0·0002 | 1·0418±0·0003 | 1·0442±0·0003 |
| Chronic hypertension | 5 | 1·0455±0·0001 | 1·0456±0·0002 | 1·0444±0·0001 | 1·0439±0·0001 | 1·0434±0·0003 | 1·0466±0·0003 |

Mean Values±SEM. * p < 0·05, † p < 0·01, ‡ p < 0·001 for significant difference from control groups (Wilcoxon’s rank sum test). SHRSP compared to WKR, the other experimental groups compared to Control (S-D).

Abbreviations: S-D = Sprague-Dawley; WKR = Wistar Kyoto Rats; SHRSP = spontaneously hypertensive rats, stroke-prone.
control and SHRSP brains that showed an increase in tracer extravasation. Amphetamine induced protein leakage particularly in frontoparietal cortical regions in agreement with earlier description, whereas bicuculline-treated rats had scattered areas of extravasation in the brain with predilection of diencephalic structures. Three of the five renal hypertensive rats showed a few discrete blue areas of tracer extravasation in cerebral cortex.

The specific gravities of various brain regions of the experimental groups and controls are presented in the table. Since the results showed no difference between the different groups of control rats, they were treated together. Rats subjected to acute hypertension by injection of amphetamine or bicuculline did not differ from controls except for a minor decrease in specific gravity in diencephalon in rats given bicuculline (p<0.05). Cortical and cerebellar samples from renal hypertensive rats were significantly lighter than controls (p<0.001). Assuming the oedema is water, the increase in tissue volume required to produce a given decrease in specific gravity (sp gr) can be calculated by the following equation:

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\text{percentage of change of tissue volume} = \frac{(\text{sp gr control} - \text{sp gr brain}) \times 100}{\text{sp gr control}}
\]

The specific gravity in cortical regions of renal hypertensive rats indicates 3.9 and 3.7% increase in volume in frontal and parietal cortex respectively. No increase in water content was observed in SHHSP. In fact, the brain stem of these rats had slightly higher specific gravity than WKR controls.

Discussion

Our results confirm some earlier reports that protein leakage in acute hypertension and experimental seizures may be associated with no or only minor increase in water content. Quantitative data on the extravasation of 125I-labelled serum albumin in various types of acute and chronic hypertension have been presented elsewhere. The most extensive extravasation occurs in connection with the acute hypertensive reaction induced by bicuculline or amphetamine. The increased permeability in the brain is slight and not consistently present in renal hypertensive rats. SHR are largely protected as to albumin leakage at high pressure levels, probably due to structural adaptation of arterial vessels. Protein leakage has been observed in SHRSP, but whether this occurs before or after clinical symptoms of stroke is controversial. Our colony of SHRSP develop high blood pressure of a similar magnitude as in Japanese laboratories but have a lower incidence of stroke. The present results with no macroscopic visible extravasation in non-perfused brains does not rule out that a minor leakage may be present. A slight leakage of endogenous serum albumin has been demonstrated in SHRSP with immunoelectrophoresis without concomitant macroscopic Evans blue-albumin extravasation (unpublished observations). The specific gravity in stroke-resistant SHR has been reported not to differ from controls, although no figures were given. The reason for the slight increase in specific gravity in the brain stem in SHRSP compared to WKR in the present study remains to be elucidated.

With an immunoelectrophoretic technique, the amount of endogenous serum albumin 15–30 minutes after bicuculline administration has been found to be 200–300 ng/mg brain tissue (wet weight) in the diencephalon and mesencephalon and 60–220 ng/mg in the cortex. This amount of albumin per se has a minor influence on the osmotic pressure of the extracellular fluid, and the effect on brain water will largely be determined by concomitant changes in electrolytes. No direct comparison can be made between the larger protein extravasation in acute hypertension and the smaller but supposedly more longlasting leakage in renal hypertension since the clearance rate of albumin from the tissue is not known. Degradation products of albumin will increase osmotic pressure more than albumin itself, and it has been suggested that 125I-labelled serum albumin is rather rapidly degraded in brain tissue. The fate of endogenous serum albumin in the brain is still largely unknown, and, in addition to local degradation, various mechanisms for removal of albumin from the extracellular fluid in the brain have been suggested such as clearing via bulk flow into the cerebrospinal fluid, uptake into astrocytes and back-transport over the cerebral capillaries.

Our data point to differences as to the development of cerebral oedema in renal and spontaneously hypertensive rats. The two models of chronic hypertension differ also with regard to the capacity of the BBB to withstand high levels of intraluminal pressure, but whether the difference in permeability per se can explain the increased tendency to develop brain oedema in renal hypertension remains to be shown. Having one intact kidney and no clinical symptoms, the rats are not likely to have been uraemic. The serum content in serum of nine renal hypertensive rats...
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treated in the same way as in the present series was not significantly higher than in controls eight weeks after the constriction (unpublished observations). Moreover, there is no evidence that uraemia alone increases the water content in the brain.26 27 In a model of renal hypertension with unilateral constriction of the renal artery and contralateral nephrectomy, Byrom28 observed increased brain water content in the cortex only in rats with macroscopic protein leakage and clinical symptoms. The more sensitive method for detecting oedema used in the present study might explain the difference. That brain oedema may occur in the absence of clinical symptoms in chronic hypertension is in agreement with the report by Rosenblum et al29 who found increased water content in unilaterally nephrectomised rats treated with DOCA and salt. It has been reported that unilateral nephrectomy in monkeys increases brain water even in the absence of hypertension and uraemia30 but the fact that the remaining kidney was wrapped in cellophane in that study might have been more important than the nephrectomy even if no hypertension developed. The present results indicate that nephrectomy is not a prerequisite for the development of brain oedema in renal hypertension.

The pathogenesis of the clinical syndrome of hypertensive encephalopathy is still not fully understood although the syndrome is correlated to increased cerebrovascular permeability and cerebral oedema.18 25 28 It is of interest in this context that the syndrome is more common in patients with renal than with primary (essential) hypertension. Whether this is correlated to a lower degree of structural adaptation of cerebral vessels in renal hypertension or to some additional factor is at present not clear. Some studies suggest a possible role of humoral factors in the pathogenesis of increased permeability and metabolic disturbance in the brain in renal hypertension.16 31 32 However, further studies are needed to clarify the mechanisms behind brain oedema in renal hypertension.

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References