on direct effects of the inhibitory substances on these routes or be secondary to the inhibition of the sodium pump.
Both ouabain and NF were shown to raise the intracellular sodium activity, accounted for by the inhibition of the sodium pump[14] which activates sodium feedback mechanisms involving the apical membrane, and hence, reduces the apical entry of sodium. NF and ouabain inhibited the sodium pump to the same extent[15], but had different effects on tissue resistance (fig. 2). These differences may reflect a greater inhibitory effect by the former on apical sodium entry and/or on paracellular pathways. To conclude, the present data demonstrate that, in toad bladder preparations, ouabain and NF act mostly by inhibiting the sodium pump; NF has an additional inhibitory effect more pronounced than that of ouabain on apical permeability to sodium and/or on the paracellular pathways for sodium transport, and NH_4Cl exerts its effect mostly on the apical membrane permeability.

Acknowledgment. This work was supported by a grant (Nr. 3.496.083) from the Swiss National Science Foundation. We would like to express our thanks to Mrs M. Gourjon and G. Lucot for their technical assistance, and Dr Richard James for reviewing the English.

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Kinins in cerebrospinal fluid: Reduced concentration in spontaneously hypertensive rats

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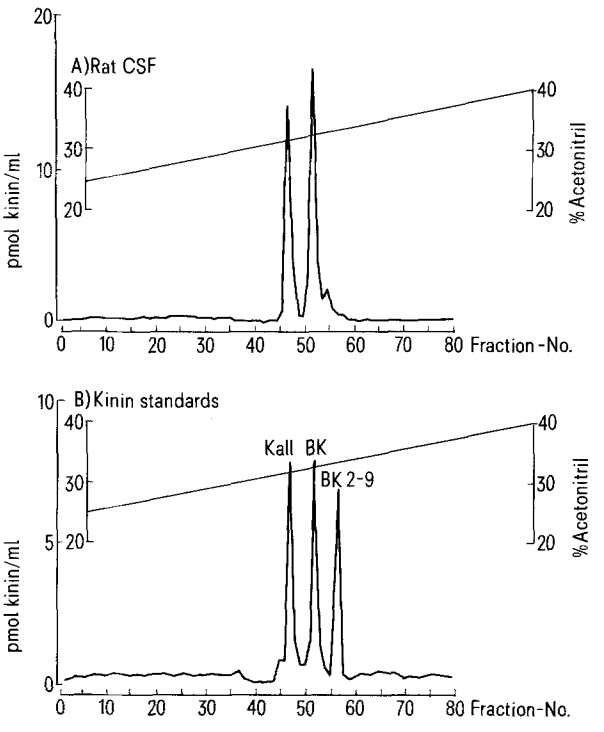
Summary. Rat cerebrospinal fluid contains peptides which displace radiolabeled bradykinin from its specific antibodies. Two peptides which showed the same retention time as kallidin and bradykinin in a reverse phase high pressure liquid chromatography system were detected in cerebrospinal fluid of rats. The concentration of radioimmunologically detected kinins in the cerebrospinal fluid of spontaneously hypertensive rats of the Okamoto strain was lower than that of the Wistar Kyoto control rats.
Key words. Kinins; bradykinin; kallidin; cerebrospinal fluid; HPLC; hypertension.

The cerebrospinal fluid (CSF) of normal rats contains a substance which has an immunoreactivity indistinguishable from that of kinins[1]. However, it is unknown whether bradykinin, kallidin or methionyl-lysyl-bradykinin is the peptide involved. To identify which kinin is present in rat CSF we used a high pressure liquid chromatography system (HPLC), in which the kinins as well as fragments of these peptides showed different retention times.
Although the i.v. (or i.a.) injection of kinins induces a fall in blood pressure, the administration of these peptides into the cerebral ventricles has the opposite effect[2]. In theory, constantly elevated levels of cerebral kinins could lead to a permanent rise of blood pressure and thus be involved in the pathogenesis of some forms of hypertension. To test this possibility we also measured the kinin concentration in the CSF of spontaneously hypertensive rats.
Materials and methods. Female normotensive Wistar Kyoto (WKR) and stroke-prone spontaneously hypertensive rats (SHRsp) of the Okamoto strain, weighing about 200 g were used. CSF (approximately 0.2 ml per rat) was collected under pentobarbital anesthesia by puncturing the Membrana atlanto-occipitalis. EDTA (12.5 mM) and o-phenanthroline (2.6 mM) were used (20 µl/ml CSF) to prevent peptide degradation.
The CSF from 25 animals was pooled and an aliquot (0.3 ml) was taken for measuring the basal kinin concentration by radioimmunoassay. The kinins, which were contained in 4.7 ml CSF, were prepurified and concentrated on octadecasylilsilica cartridges (3; Sep-Pak C_{18}, Waters, Eschborn, Federal Republic of Germany). Prior to sample application the cartridges were first rinsed with 3 ml methanol and then with 5 ml water. After the CSF was applied to the cartridges, these were rinsed with 9 ml 4% acetic acid in water. Then the peptides were eluted with 3 ml isopropanol. The isopropanol was removed from the eluate with a stream of nitrogen while the test tubes were immersed in a water bath at 40 °C. The remaining water phase was lyophilized. The freeze-dried residue was dissolved in 1 ml 0.001 N HCl. A sample of 0.3 ml was taken to assess the recovery of kinins, and the rest was applied to the HPLC column. The column was calibrated with synthetic kallidin and bradykinin. The peptides were detected by UV extinction at 220 nm and 0.1 absorbance units full scale.

The high pressure liquid chromatograph was carried out at room temperature on a 300 × 4 mm reverse phase C_{18} column (µ-Bondapak C_{18}, 10 µm, Waters). The mobilie phases were passed through a RC 55 membrane filter with 0.45 µm pore size (Schleicher and Schüll, Dassel, Federal Republic of Germany) and degassed under vacuum before use. The column was first rinsed with 40% acetonitrile in 0.08% (v/v) trifluoracetic acid (solution B), and then equilibrated with 20% acetonitrile in 0.08% trifluoracetic acid (solution A) for another 15 min. A linear gradient from solution A to solution B at a flow rate of 1 ml/min was started 3 min after sample application and completed 45 min later. Fractions of 0.5 ml were collected in polyethylene tubes. The samples were dried as described for the eluates of the Sep-Pak cartridges, and the residue dissolved in



Characterization of the kinins present in rat cerebrospinal fluid. CSF kinins were separated with a reverse phase μ-Bondopak C_{18} column in an HPLC system using an acetonitrile gradient for the elution. Measurements of kinins in the eluates was carried out with a RIA using an antibody which is specific for bradykinin and related peptides. *A* Separation of endogenous kinins in rat CSF. *B* Native rat CSF with added synthetic kallidin, bradykinin and the bradykinin fragment 2–9. Two substances could be identified in rat CSF. The first one co-eluted with kallidin whereas the second had a retention time identical to that of bradykinin.

0.1 ml radioimmunoassay (RIA) buffer (Tris/HCl 0.1 M, pH 7.4, containing 0.1% gelatine, 0.1% neomycin and 1.2 mM o-phenanthroline). Antibodies obtained by repeatedly injecting a rabbit with synthetic bradykinin coupled to human serum albumin by 1,5-difluoro-2,4-dinitrobenzene, were used to measure the kinin concentration in the elution fractions from the HPLC by radioimmunoassay[4]. In a separate run 1 ml of CSF to which 10 ng kallidin, 10 ng bradykinin and 10 ng des-arg-1-bradykinin had been added, was prepurified and chromatographed in the same manner. The above mentioned RIA was also used to quantitate the kinins in the CSF from WKR and SHRsp.

Results. The concentration of kinin-like immunoreactivity in pooled native CSF of WKR was 0.88 ng/ml. Thus, 4.14 ng kinins were applied to the octadecasylilsilica cartridge. Only 2% of the added kinins were lost during this purification and concentration step. Part of the eluate (2.85 ng kinins) was applied to the HPLC column (The rest was used for kinin measurement by RIA).

Two kinin-like peptides were detected in the fractions of the HPLC column. One of the elution peaks had a retention time identical to that of kallidin and the other one identical to that of bradykinin (fig., A). No other kinin-like peptides were detected. Simultaneous chromatography with the standards revealed the identity of the retention times of the natural peptides with that of synthetic kallidin and bradykinin (fig., B). The sum of the estimated kallidin (1.41 ng) and bradykinin (1.63 ng) eluted from the HPLC column suggests a complete recovery (106%) of the added kinins during this step. Based on an estimated overall recovery of 100%, it can be calculated that the kallidin concentration in native cerebrospinal fluid is 0.40 ng/ml and that of bradykinin 0.48 ng/ml.

The kinin concentration in native CSF from WKR was 0.81 ± 0.07 ng/ml (n = 8) whereas that in CSF from SHRsp was 0.54 ± 0.09 ng/ml (n = 5). Student's t-test revealed that this difference was significant ($p < 0.05$).

Discussion. Kinin releasing enzymes (kininogenases) have been found both in total cerebral homogenates and in homogenates from the hypophysis[5,6]. Kinins have been identified in the central nervous system of the rat[7,8]. This strongly suggests that the central nervous system has the capacity to produce kinins. We could not only confirm the previous observation of Thomas et al.[1], that CSF contains immunoreactive kinins, but in addition identified them as kallidin and bradykinin.

These kinins could either be locally produced or reach this space through the blood-brain barrier. The last possibility is unlikely since i.v. administered radioactive bradykinin does not seem to accumulate in the brain[9]. The finding of kallidin in CSF suggests that these peptides are released by a cerebral kininogenase. Kallidin is the peptide released by tissue but not by plasma kallikrein from kininogen. A kallikrein-like enzyme has been reported to occur in pituitary glands[6,10]. Also tissue kallikrein has been localized by immunohistochemistry in brain ventricular epithelium and hypothalamic cells[11].

It is uncertain what role the kinins play in the central nervous system. These peptides might be involved in nociceptive transmission or in the regulation of blood pressure[2,12,13].

Since the CSF kinin concentration of SHRsp was lower than that of WKR, these peptides are probably not directly involved in the pathogenesis of the hypertension. It might be hypothesized that the reduced CSF kinin concentration of the SHRsp rats reflects a compensatory mechanism to an elevation of blood pressure due to an abnormality in other systems.

Acknowledgments. The technical assistance of F. Bles and J. Kopatsch is acknowledged. This work was supported by the German Research Foundation (SFB 90).

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