Ionized Calcium in the Effluent from Ventriculo-Cisternal Perfusion in the Rabbit

There has been some controversy concerning the problem whether the calcium concentration in the cerebrospinal fluid (CSF) is influenced by the calcium concentration in the plasma when the latter is altered by experimental procedures. KEMÉNY et al.[1,2] did not find any change after 5–6 h of continuous i.v. infusion of calcium and cisternal drainage of CSF in dogs in larger series of experiments. SCHAIN[3] in similar experiments reported a distinct rise in the CSF concentration of calcium of two dogs after 2 h and BRADBURY[4] found a minor rise of the calcium concentration in the effluent during perfusion of the ventricular system of rabbits with a high concentration of calcium in plasma, maintained for 2 h.

No previous studies seem to have been undertaken on the concentration of ionized calcium in CSF when the concentration of ionized calcium or the pH of the plasma is varied.

Methods. Rabbits anaesthetized with pentobarbital-sodium (initial dose: 30 mg/kg i.v.) were used. The preparation of the animal for ventriculo-cisternal perfusion was that described by OLDENDORF and DAVSON[5], always performed bilaterally and for 3 h. A steady state of concentrations in the cisternal effluent was achieved after 75 min and only results after this time are included. The perfusion solution was that described by MERLIS[6] with the modification that glucose was replaced by urea. Inulin (80 mg/100 ml) was added to the solution, allowing the estimation of CSF secretion rate as originally described by HEISEY et al.[7].

Blood-samples were obtained from the femoral artery. In some experiments the concentration of ionized calcium in the plasma was brought to a new constant level by infusions via the femoral vein. The infusion solutions were: Isotonic $CaCl_2$, 100 mM Na_2EDTA in isotonic NaCl, approximately 100 m*M* choline-EGTA. In 2 experiments a solution of 1 *M* NH_4Cl was infused. The infusions were stopped if the blood pressure fell or bradycardia occurred and started again when the situation had been normalized. Perfusion was started when the infusion had been going on for $1^1/_2$ h. The concentration of ionized calcium in serum and in the cisternal effluent was measured using the method described by PEDERSEN[8–10], involving production of serum-ultrafiltrates at 37°C and P_{CO_2} = 40 mmHg and colorimetrical determination with tetramethylmurexide. In all of the experiments, the pH of the retained serum after ultrafiltration was measured. In about one third of the experiments, the pH of the blood was measured. pH in the cisternal effluent was not measured due to the necessary use of silicone-tubes including the possibility of CO_2-escape. Inulin was measured colorimetrically as fructose using anthrone following acid hydrolysis (according to a modification by SKADHAUGE et al. of the method of HILGER et al.[11]). As double determinations were not possible, single determinations of ionized calcium or inulin which differed more than 3 times SD in that experiment after steady state were excluded from the data.

Results. Both increasing and decreasing the serum concentration of ionized calcium from approximately 1.50 to approximately 3.00 mmoles/l, respectively 1.00 mmoles/l, caused the concentration in the cisternal effluent to rise from the normal approximately 0.95 to approximately 1.10 mmoles/l. In 2 experiments (one with a very slow infusion of $CaCl_2$) the cisternal effluent concentration was high in spite of normal values of serum concentration (Figure 1). In 2 experiments NH_4Cl was infused i.v. in order to test the effect of pH. These results are also shown in Figure 1.

Figure 2 shows the relationship between the cisternal effluent concentration and the pH in serum after ultrafiltration in 23 experiments. A decrease of pH increases the concentration of ionized calcium in the cisternal effluent. Inspection of the data showed that this relation was not influenced by the concentration of ionized calcium in serum. The phenomenon was also seen in 9 experiments in which the actual pH in the blood was measured. In these experiments the pH after ultrafiltration was 0.05–0.15 pH units lower than that of the blood-samples, the difference being greater the more acid the blood.

Discussion. The blood-cerebrospinal barrier has been found to be permeable to calcium[12–15]. However, in the

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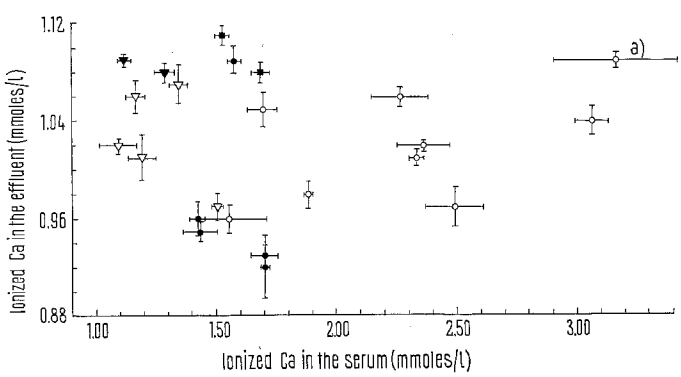

Fig. 1. The concentration of ionized calcium in the effluent from the ventriculo-cisternal perfusion system as a function of the concentration of ionized calcium in the serum. Each point represents the average of 2–6 measurements in 1 experiment. Vertical and horizontal bars indicate ± S.E.M. (23 experiments on 23 rabbits). Symbols: ●, no infusion; ○, $CaCl_2$ i.v.; ▽, Na_2EDTA i.v.; ▼, Choline-EGTA i.v.; ■, NH_4Cl i.v. a) The 1 cannula after the experiment was found to be placed in the subarachnoid space in stead of in the ventricle.

present experiments fairly large changes in the serum concentration of ionized calcium were only to a small degree reflected in the effluent from the perfusion system.

The difference between pH in the serum after ultrafiltration and in the blood probably reflects that serum from the more or less metabolically acidotic animals during the ultrafiltration procedure was equilibrated with a $P_{CO_2} = 40$ mm Hg.

The marked effect of pH may be explained in different ways: A pH induced increase in serum concentration in ionized calcium can hardly have been of a magnitude to cause the observed change, provided that the permeability from blood to CSF has not changed considerably.

Changes in the secretion rate of CSF may explain part of the effects observed. However, GRAZIANI et al.[15] have previously demonstrated that only part of the Ca-flux from plasma to CSF was affected by the secretion rate, and inspection of the data from the present experiments showed no tendency at all towards direct proportionality between the concentration of ionized calcium in the cisternal effluent and the rate of secretion of CSF.

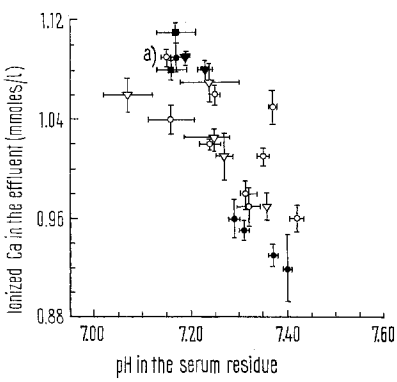

Fig. 2. The concentration of ionized calcium in the effluent as a function of the pH in the serum residue after ultrafiltration. Symbols as in Figure 1 (23 experiments on 23 rabbits).

It is known that pH affects the binding of calcium to subcellular structures, for instance in liver. Calcium is also bound to cerebral tissue[16]. An interference with this calcium binding by a decrease of pH may explain the observed increases in ionized calcium concentration in the effluent, provided that pH in the latter changed in the same direction as pH in plasma.

However, if a simple change in binding conditions took place, as a consequence of a change of pH, one would not expect this to cause the observed constant increase of ionized calcium concentration in the cisternal effluent, but rather an initial sudden change followed by an approach to a constant concentration.

Calcium has been shown to be transported between the extracellular and intracellular compartments in the brain, for instance through sodium-calcium exchange, demonstrated in cerebral cortex from cats[17]. An interference of pH with such transport-mechanisms may also explain the observed effects of pH.

The possible physiological significance of this phenomenon awaits exploration.

The results also suggest that in studies of calcium transport into and out of the CSF the pH of this fluid and blood should be rigorously controlled.

Zusammenfassung. Es wird am Kaninchen gezeigt, dass die Konzentration ionisierten Kalziums im Ausfluss eines ventriculo-cisternalen Perfusionssystems nur wenig von der Plasmakonzentration beeinflusst ist. pH-Erniedrigung im Blut erhöhte die Konzentration erheblich.

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Stimulation by Caffeine of the Calcium Efflux in Barnacle Muscle Fibers

There is evidence that the contraction of skeletal muscle caused by caffeine is associated with little or no depolarization of the fiber membrane[1–3]. Contraction is explained as being due to the release by caffeine of internally 'bound' calcium[4,5]. There also is evidence that caffeine stimulates ^{45}Ca efflux in frog and crab muscle[6,7], and ^{89}Sr efflux in barnacle muscle fibers[8]. The purpose of the following communication is to describe the kinetic results obtained by loading single barnacle fibers with radiocalcium and to show that caffeine, whether applied externally or internally, causes a rise in the Ca efflux.

Single muscle fibers were isolated by dissection from the depressor muscle bundles of specimens of *Balanus nubilus* or *B. aquila*. Fibers were cannulated in the same way as crab muscle fibers[3] and were then loaded with ^{45}Ca by means of a microinjector[9] as modified by CALDWELL and WALSTER[3]. The composition of the bathing fluid used was as follows (m*M*/l): NaCl 465, KCl 10, $CaCl_2$ 10, $MgCl_2$ 10, $NaHCO_3$ 10 and pH 7.8. Caffeine was obtained from Sigma Chemical Company.

The activity of ^{45}Ca in the wash-out specimens and the activity remaining in the fiber at the end of the experiment were assayed with plastic phosphor scintillators (Nuclear Enterprises 102A). The thickness of the wall of the phosphor cell (outer) was 3 mm. The solid 'inner' phosphor was designed so as to displace the 1.2 ml of wash-out sample, leaving only a fine film of fluid lying in

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