Failure to Demonstrate the Presence of a Sodium Transport Inhibiting Factor in the Serum of Uremic Patients

Despite suggestive evidence of the presence of a normally occurring natriuretic hormone[1], the isolation and positive identification of such a hormone remains to be accomplished. Arguing that the concentration of this factor, if present, would be expected to be highest where appreciable sodium loads are excreted by minimal renal tubular mass, BOURGOIGNIE et al.[2] sought, and claim to have found, a sodium transport-inhibiting effect in the sera of uremic subjects on minimally restricted dietary sodium intake. We have been unable to confirm their results.

Method. Sera were obtained from 7 hospitalized adults and 4 control subjects; the latter were normal adult male laboratory workers, all on unrestricted sodium intake and in apparently good health. Of the 7 patients, 5 had chronic renal insufficiency of several years' standing; their blood ureas ranged between 235 and 280 mg/100 ml and their creatinine clearances between 1 and 13.5 ml/min. 4 of these patients were on infrequent peritoneal dialysis, while the 5th received maintenance machine dialysis thrice weekly. The 6th patient had acute tubular necrosis, of unknown cause; his blood urea was 228 mg/100 ml. The diagnosis of the 7th patient, whose blood urea was 160 mg/100 ml, was not established. None of the patients was on restricted sodium intake. In those patients on maintenance dialysis, all blood samples were taken before dialysis.

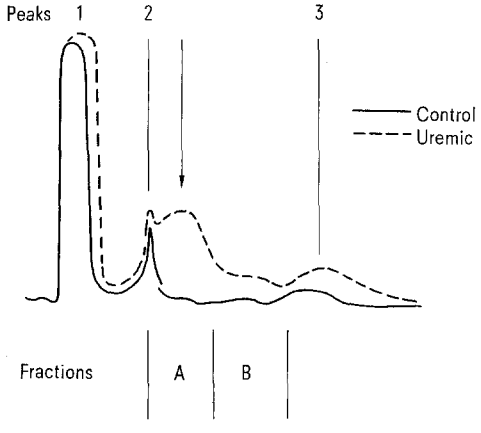

Fig. 1. Typical UV-absorption pattern (280 nm) of eluants of control (solid line) and uremic (dashed line) sera. The arrow indicates the additional deflection seen in the uremic sera. The volumes of eluant comprizing the fractions A and B are indicated below.

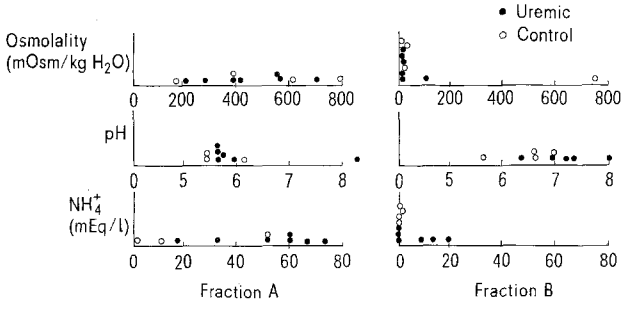

Fig. 2. Distribution of osmolality, pH and NH_4^+ concentrations in eluant fractions A and B in both control and uremic sera.

All blood samples were processed as described by BOURGOIGNIE et al.[2]. The sera were fractionated by Sephadex-Gel filtration. The UV-absorption patterns of successive fractions exhibited 3 peaks, (Figure 1) labelled here 1, 2 and 3. The samples between the apex of peak 2 and the return of the trace to the baseline were pooled and labelled fraction A; those beyond this point, up to, but not including, peak 3, were pooled and labelled fraction B; this fraction corresponds to that said by BOURGOIGNIE et al. to contain a sodium transport-inhibiting factor. Osmolality, pH and NH_4^+concentrations were measured in all fractions[3].

The effects of the A and B fractions on active sodium transport were then assayed using the isolated abdominal skin of the South African clawed toad (*Xenopus laevis*) and, in some instances, the isolated and perfused proximal convoluted tubule of the rabbit kidney[4,5]. In the former the short-circuit current, and in the latter the rate of fluid reabsorption, was equated with the degree of active sodium transport. The fractions were added to the fluid bathing the inner surface of the toad skin, or to the peritubular fluid. The final concentration of the A fraction was 1/40 to 1/20, while that of fraction B was identical to that pertaining in the original serum. 4 tubules were exposed to the A fractions obtained from the sera of 2 uremic patients with chronic renal insufficiency, and 1 tubule to that from a normal control subject.

Results. The UV-absorption patterns of our uremic subjects differed from those of the controls, and from those described by BOURGOIGNIE et al.[2], in that all displayed an additional deflection immediately after the second peak (Figure 1). The height and extent of this additional deflection bore no obvious relationship to the blood urea concentration in the individual patients.

The osmolality, pH and NH_4^+ concentrations of the A and B fractions were very different, although no obvious differences were observed between control and uremic subjects in either fraction (Figure 2). The mean osmolar and NH_4^+ contents of the B fractions were negligibly low, but the pH was higher than that of the A fraction.

Two of the 4 fractions obtained from the controls, and all 7 derived from the uremic sera, produced a fall in short-circuit current (mean 16.9%, SD 14.6%). Only one of the B fractions (from a patient with chronic renal insufficiency) similarly lowered the short-circuit current (by 20%); this sample, however, also had a very high NH_4^+ concentration (18 mM l^{-1}).

Each of the 5 tubules exposed to an A fraction showed an apparent increase in fluid reabsorption rate, as judged by a fall in the volume of fluid emerging from the distal end of the tubule at a constant perfusion rate (mean 26.7%; SD 7.1%).

Discussion. Only one of the B fractions, from our 7 uremic subjects, produced a fall in short-circuit current, and this particular sample had an exceptionally high NH_4^+ concentration. We are thus unable to confirm the suggestion of the presence of a sodium transport-inhibiting

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substance in the sera of uremic patients[2]. Perhaps the proposed substance was present in our A fractions. As these were rich in salts and of low pH (Figure 2), we diluted them considerably before adding them to the solutions bathing the toad skins and tubules; we hoped to minimize the 'salt effects' while retaining evidence of inhibition of sodium transport, as might occur were the inhibitor initially present in high concentration. The diluted A fraction did inhibit sodium transport in 2 of the 4 controls, and in all of the 6 uremics tested, but the degree of inhibition was commensurate with that expected simply on the basis of changing the osmolality, NH_4^+ concentration and decreased pH of the bathing solution, all of which factors are known strongly to inhibit active sodium transport[6–8].

It might be expected that mammalian, and particularly renal, tissue would be more sensitive to the presence of the postulated inhibitor than amphibian tissue. Far from this being the case, the extracts produced increased rather than decreased, fluid reabsorption across the proximal tubular wall. We believe this reflects the presence of induced tissue damage, with resultant increased passive permeability, rather than an increase in active sodium transport. We have thus been unable to demonstrate the presence of a factor in the sera of uremic patients capable of inhibiting sodium transport in either toad skin or isolated and perfused proximal tubules of the rabbit kidney.

Zusammenfassung. Die Wirkung verschiedener Serumfraktionen von Urämikern wurde mit Sephadex-Gelfiltration auf den Natriumtransport an der Krötenhaut am isolierten proximalen Tubulus von Kaninchen untersucht. Eine Hemmung des Natriumtransportes konnte nicht festgestellt werden.

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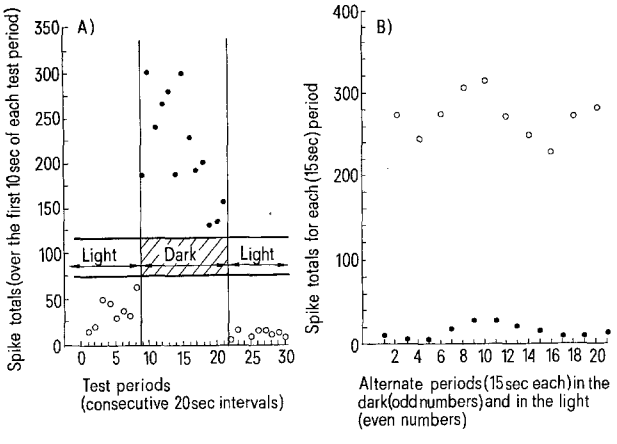
Is There a Single Cell Code for Background Light Levels?

When recording from single cells in the visual pathways, one occasionally encounters units, particularly in the midbrain, which give no response to localized light changes[1], yet their level of maintained discharge[2] does change over the longterm in a consistent way with the luminance of the environment. Could these units be part of a system which monitors and conveys luminance information to 'analyser' sites perhaps related to such activities as pupil control and adaptation state reference?

The following observations support the presence of such a coding system, and show in the midbrain how luminance changes in the visual surround can be demonstrated as a function of maintained activity at the single cell level.



Two midbrain cells with no spatially demonstrable receptive fields, but whose rates of maintained activity altered with changes of background luminance: A) a cell which increased its discharge rate in darkness; B) a cell which decreased its discharge rate in darkness. Dark (0.03 cd/m²) period responses are shown as filled circles; light (8 cd/m²) period responses are shown as open circles.

The examples cited are from amongst a cumulative sample of nearly 300 neurons within the rabbit mesencephalon now studied. The animals were prepared under light urethane anesthesia (3.3 to 6.0 ml/kg body weight of a 20% solution in saline, a dosage level found from earlier work to be no more detrimental to cell responsiveness than 'encéphale isolé' techniques). This was supplemented with 3.3 mg/kg body wt./h of gallamine triethiodide to prevent eye and body movements, the animal being artificially respired during that period.

The animals were supported stereotaxically, the stimulated eye in each case being refracted and fitted with a contact lens to protect the cornea from drying and to bring the retina into conjugacy with the 57 cm testing plane. Stainless steel microelectrodes (average resistance, 40 megohms) were introduced into the superior colliculus through an agar sealed skull aperture. Recording sites were later verified with the Prussian blue reaction.

Each cell was tested for the presence of a spatially defined receptive field. The particular cells of interest here, however, were those having no demonstrable receptive field to transient stimuli, but whose maintained background activity would change with time constants of seconds or even minutes to differential levels of background luminance.

The Figure shows the temporal histories of 2 such midbrain cells, giving no response to spatially limited stimuli, but marked response to changes of background luminance. In graph A), the maintained activity levels of a stratum opticum cell in the superior colliculus were integrated over alternate 10 sec periods, first in a bright environment (8 cd/m²), then in a very dim environment (0.03 cd/m²), and then in the bright environment once again. A considerable increase in dark period activity can

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