

References and Notes

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Accumulation of Potassium Anaerobically by Renal Medullary Slices

Abstract. Potassium accumulation occurred in leached slices of the inner medulla of the dog kidney incubated under anaerobic conditions at 37°C with glucose as substrate, but was blocked by inhibitors of glycolysis. Aerobically, only slight accumulation occurred with succinate as substrate at 37°C, and this was blocked by dinitrophenol. These findings were in contrast to those obtained with kidney cortex slices under the same conditions.

Recent work from this laboratory (1) showed that tissue slices of the inner zone of the medulla of the dog kidney have a high rate of anaerobic glycolysis and a low rate of oxygen consumption when compared to slices of the cortex of the kidney. It was further found that increasing the osmolality of the medium by addition of sodium chloride caused no diminution in the rate of glycolysis of the medullary slices until the osmolality exceeded 1100 to 1300 mosm/kg of water; the cortical slices, on the other hand, were inhibited progressively as osmolality was increased above the control level of 300 mosm/kg of water. Respiration was similarly inhibited in both types of slices by increasing osmolality.

These findings, considered with other evidence derived from physiological investigations, suggest that the renal medulla *in situ* derives its energy in a milieu of high osmolality mainly from anaerobic pathways. In view of the role of the loops of Henle and the collecting ducts in the countercurrent mechanism for urinary concentration, we have conducted studies concerned with the identification and characterization of active transport systems for ions in this tissue. In the course of these studies, a novel type of potassium accumulation has been found, an accumulation that appears to be dependent upon anaerobic rather than aerobic metabolism. This report is based on studies of this system

and compares it to the system for potassium accumulation in the kidney cortex previously described by Mudge (2) and by Whittam and Davies (3).

Tissue slices of the dog kidney cortex and inner medulla, prepared as described elsewhere (1), were incubated in Krebs-Henseleit bicarbonate medium without substrate for 30 minutes at 0°C and gassed with either N₂-CO₂ or O₂-CO₂ (see Table 1). These leached slices were then transferred to fresh Krebs-Henseleit bicarbonate medium containing substrate and reincubated for an additional 45-minute period under conditions shown in Table 1. Then the slices were removed, blotted, weighed, and dried. Potassium was measured on nitric acid digests of the tissue and on the medium by internal standard flame photometry.

Table 1 shows that leached slices of the inner medulla reaccumulate potassium when incubated under anaerobic conditions (at 37°C) with glucose as substrate. This reaccumulation of potassium, although it never reached *in vivo* levels, was blocked by iodoacetate, fluoride, or ouabain, or by carrying out the incubation at 0°C. Pitressin had no effect. Increasing the osmolality to 1.02 or to 2.10 osm/kg of water by addition of sodium chloride blocked reaccumulation. This effect is probably

not mediated specifically through failure of energy metabolism, since previous studies (1) have shown that osmolalities in the range of 1.0 osm/kg of water do not inhibit anaerobic glycolysis in medullary slices.

When leached slices of the inner medulla were incubated aerobically with succinate as substrate they showed only slight accumulation of potassium compared with that observed anaerobically. Further losses of potassium occurred from leached slices incubated at 0°C, at 37°C, in the presence of dinitrophenol, or greatly increased osmolality.

Comparative studies of slices of the kidney cortex showed a different pattern of behavior from that observed in the inner medullary slices. Anaerobic incubation of leached cortical slices caused a loss of potassium; iodoacetate, fluoride, and high osmolality all increased the loss, while ouabain and Pitressin had no effect beyond that observed for the control. When the incubation was carried out anaerobically at 0°C, the loss was less than that observed at 37°C.

Aerobically the results confirm those previously reported by others (2,3). Reaccumulation of potassium occurred in the complete medium at 37°C but not at 0°C. Losses of potassium occurred from leached slices in the presence of dinitrophenol or increased osmolality.

Table 1. Influence of various factors upon potassium reaccumulation in leached slices of inner medulla and cortex of the dog kidney. The slices were leached in Krebs-Henseleit bicarbonate medium without substrate at 0°C for 30 min. Further incubations were then carried out in a complete medium composed of Krebs-Henseleit bicarbonate medium with 10mM glucose (anaerobic incubations) or 10mM succinate (aerobic incubations). The potassium concentration of the complete medium was 6 to 7 meq/lit. Gas phases were 5 percent CO₂ in either 95 percent O₂ or N₂ at 37°C and 2.8 percent CO₂ in either 97.2 percent O₂ or N₂ at 0°C. Inhibitor concentrations were: iodoacetate, $1.7 \times 10^{-3}M$; sodium fluoride, $5 \times 10^{-2}M$; ouabain, $10^{-6}M$; Pitressin (Parke Davis), 0.2 unit/ml; dinitrophenol, $2 \times 10^{-4}M$; osmolality of the medium was increased by addition of sodium chloride. The concentration gradient for potassium was computed as follows: ($\mu\text{mole K}^+/\text{g of tissue water}$) $\div$ ($\mu\text{mole K}^+/\text{ml of medium}$). No appreciable change in the pattern of the data results if the contribution of the extracellular space is neglected in this calculation. The gradient for fresh tissue was calculated by using the determined values for serum potassium. Data shown represent mean and standard deviation; the numbers in parentheses represent the number of experiments.

Conditions	Concentration gradient for K ⁺	
	Inner medulla	Cortex
Fresh tissue	9.91 $\pm$ 0.35 (21)	17.50 $\pm$ 0.40 (15)
After leaching at 0°C, gassed with N ₂ -CO ₂	2.21 $\pm$ 0.10 (11)	6.15 $\pm$ 0.59 (11)
Further anaerobic incubations:		
At 37°C	3.66 $\pm$ 0.13 (9)	2.67 $\pm$ 0.05 (12)
At 0°C	1.73 $\pm$ 0.13 (6)	5.57 $\pm$ 0.37 (4)
With iodoacetate	1.42 $\pm$ 0.03 (3)	1.71 $\pm$ 0.04 (3)
With fluoride	2.25 $\pm$ 0.02 (3)	1.88 $\pm$ 0.05 (3)
With ouabain	1.91 $\pm$ 0.04 (3)	2.83 $\pm$ 0.07 (2)
With Pitressin	3.86 $\pm$ 0.10 (3)	2.70 $\pm$ 0.09 (3)
At 1.02 osmolal	1.38 $\pm$ 0.07 (3)	2.23 $\pm$ 0.14 (2)
At 2.10 osmolal	1.38 $\pm$ 0.06 (3)	1.43 $\pm$ 0.06 (3)
After leaching at 0°C, gassed with O ₂ -CO ₂	2.22 $\pm$ 0.03 (2)	6.25 $\pm$ 0.55 (2)
Further aerobic incubations:		
At 37°C	2.63 $\pm$ 0.10 (2)	14.10 $\pm$ 0.70 (2)
At 0°C	1.98 $\pm$ 0.17 (2)	4.00 $\pm$ 0.14 (2)
With dinitrophenol	1.65 $\pm$ 0.14 (2)	2.93 $\pm$ 0.12 (2)
At 1.04 osmolal	2.28 $\pm$ 0.07 (2)	2.19 $\pm$ 0.18 (2)
At 1.68 osmolal	1.20 $\pm$ 0.01 (2)	1.49 $\pm$ 0.02 (2)

The results of these experiments demonstrate that inner medullary slices can accumulate potassium against concentration gradients and that this accumulation is dependent upon anaerobic rather than aerobic energy metabolism. EDWARD L. KEAN, PATRICIA H. ADAMS,

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4. This work was supported by U.S. Public Health Service grant No. R.G.-7106. One of us (E.L.K.) holds a Karr fellowship in biochemistry from Smith Kline and French Laboratories.

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Effects of Context on the Subjective Equation of Auditory and Visual Intensities

Abstract. Thirty-six subjects were instructed to equate the loudness of a pure tone with the brightness of an illuminated field. Eighteen of the subjects had been given brief preliminary experience with tones of low intensity; the remaining 18, with tones of high intensity. A significant and substantial effect upon equation was demonstrated.

Several recent studies have suggested that "sensory magnitude" is a self-evident dimension, susceptible of direct introspective evaluation even by inexperienced subjects (1). Such subjects are capable of consistent judgments of "sensory magnitude"; furthermore, they seem to be able to match the intensity of a sensation in one modality with that of a sensation in another in a manner consistent with their first-order judgments.

Without disputing the basic data, Warren (2) has vigorously questioned the presumption that they represent a sensory dimension, rather than an essentially perceptual continuum. Garner (3), also, has emphasized that judgments of this sort can be "reliable" without being "valid" and has demonstrated that the context in which direct judgments of sensory intensity are made can have a radical influence upon their magnitudes. We have investigated, analogously, the effects of context upon the cross-modality equation of "sensory intensities."

Thirty-six female undergraduates were divided at random into two groups ("low" and "high") of 18 members each, and each subject was tested individually in a soundproof, lightproof room. A subject of either group was seated alone in the experimental room, the experimenter maintaining contact with her by means of a Teletalk communication system. After a dark-adaptation period of 3 minutes, the subject began a series of visual judgments. At a distance of 18 inches from her eyes, she was presented with an illuminated disk, 1.5 inches in diameter (visual angle, $4^{\circ}48'$), produced by passing the beam from a 200-watt slide projector through an Eastman No. 58 Wratten ("green") filter and then through a small port in the exterior wall of the experimental room; the beam was finally used to illuminate from the rear a translucent plastic screen, set in the interior wall of the room and masked down to form the circular patch described. The intensity of the projector's beam was controlled by use of a General Radio Variac, monitored by electronic voltmeter; filtering of the beam served to prevent perceptible changes of hue in the stimulus. On each trial, the subject was first presented with a stimulus of 15 db (reference level, 0.15 mlam) for 2 seconds, and then immediately with one of either 0, 5, 10, 15, 20, 25, or 30 db for a further 2 seconds; all the timing of stimuli was accomplished automatically by a Hunter timer. The subject was asked to estimate the sensory intensity of the second stimulus on a scale which took that of the first arbitrarily as 10. Each comparison-stimulus was presented five times, in a random order unique to each subject; thus, she was required to make 35 visual judgments.

In a similar fashion, the subject next undertook 35 auditory estimations. Tones of 1024 cycles whose purity had been verified by oscilloscope were administered monaurally, with a Western Electric 6B audiometer as a source. A subject in the low group judged stimuli of either 25, 30, 35, 40, 45, 50, or 55 db (reference level, normal threshold) with respect to a standard of 40 db; a subject in the high group judged stimuli of 55, 60, 65, 70, 75, 80, or 85 db against a standard of 70 db. Again, there were five estimates at each possible level, randomly ordered; again, each standard was arbitrarily labeled 10.

As a final task, the subject performed 15 cross-modality equations. A

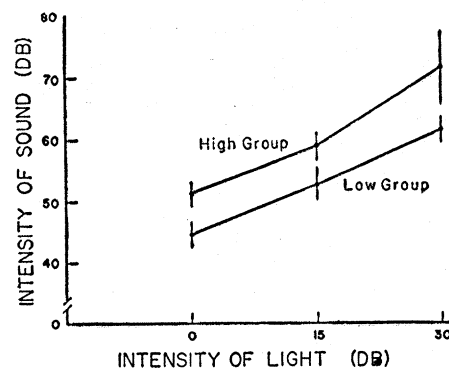


Fig. 1. Mean sound intensities required for equation with each of three light intensities, for each group of subjects ($N = 18$ per group). Vertical bars indicate $\pm \sigma$.

visual stimulus at the level of 0, 15, or 30 db was presented for 2 seconds, along with a tone of 55 db. At the subject's direction, this tone was altered in 5-db steps in additional simultaneous administrations until she was satisfied that a match in sensory intensities had been achieved. To obviate the possibility of mere "semantic matches" in this phase of the experiment, instructions emphasized that the numbers previously assigned to the stimuli were now of no significance and that they were to be ignored in making the cross-modality matches. Visual levels were ordered at random; there were five equations of sound with light at each of the three levels of brightness.

After each subject had made five judgments or five equations at each level of stimulation, the five values were averaged to provide a single score for the subject at that level. Figure 1

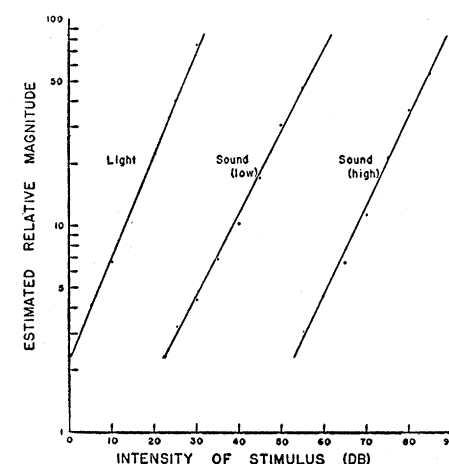


Fig. 2. Mean estimates of relative sensory magnitudes for light (function at left; $N = 36$, standard = 15 db), for sound at low level (middle function; $N = 18$, standard = 40 db), and for sound at high level (function at right; $N = 18$, standard = 70 db).