EFFECT OF FUROSEMIDE, BUMETANIDE AND PIRETANIDE ON THE SENSOR OF THE TUBULOGLOMERULAR FEEDBACK MECHANISM

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Introduction

Autoregulation of single nephron GFR is thought to depend on the integrity of chloride ion transport within the macula densa region, since the effectiveness of feedback operation is modulated considerably by intratubularly applied furosemide [1]. This fact raised the question whether feedback inhibition was a common feature of high ceiling diuretics and further how this might contribute to their mode of action. In order to analyse this problem, the feedback sensor at the macula densa was exposed to different concentrations of furosemide, bumetanide and piretanide while a maximal feedback stimulus was applied. The respective dose-feedback-response relations were determined.

Methods

Male albino Wistar rats (Han-WIST) between 180 and 225 g b.w. were anaesthetised with thiobarbital (Inactin, 120 mg/kg b.w., i.p.) and were prepared for micropuncture experiments on the left kidney in the usual way [2]. To compensate for extracellular fluid loss during the micropuncture experiments, all animals received an i.v. infusion of 1.6 ml/hr lactate Ringer's to which 3.5 μCi 3H-inulin were added per ml for clearance analysis. Initially 3.5 μCi 3H-inulin were administered as a priming dose ½hr before the first clearance period. Normal kidney function was verified by blood pressure monitoring from the right carotid artery, 3H-inulin clearance, urine flow rate, Na, K and Cl analysis of serum and urine samples and also generally by determination of lissamine green transit times. Experiments were considered as reliable when the functional parameters fell within the normal range.

Micropuncture experiments were performed on randomly selected superficial nephrons. The experimental protocol, as described elsewhere in these proceedings [3] comprised a mid-proximal tubule blockade with paraffin wax to allow simultaneously continuous stop flow pressure (SFP) monitoring in the supra-blockade tubule segment and the perfusion of Henle's loop at different flow rates without pressure transmission. Perfusion solutions consisted of isotonic
saline containing logarithmically progressing concentrations of furosemide, bumetanide or piretanide\(^+\) ranging from 10\(^{-6}\) to 10\(^{-4}\) molar. All solutions were freshly prepared from stock solutions stored at 4°C in the dark. Maximal feedback response was elicited by setting the end-proximal flow rate at 50 nl/min. The magnitude of the feedback response was defined as the SFP difference between zero and 50 nl/min loop perfusion. Control perfusates consisted of diuretic free saline solution. The data obtained from each of the experimental groups were pooled and expressed as mean ± SEM. The significance of differences was tested using the Student’s test for unrelated samples and was considered to apply when the two-tailed probability did not exceed 0.05.

Results

Zero loop perfusion yielded SFP values ranging from 25 to 35 mm Hg. At 50 nl/min loop perfusion, SFP decreased by 7.2 ± 0.3 mm Hg. Perfusion of 10\(^{-6}\) M furosemide, bumetanide or piretanide did not result in any significant change of the maximal feedback response. In contrast, feedback inhibition was complete at 10\(^{-4}\) M with each of the three diuretics. Within this concentration range, the effects of two further, logarithmically-spaced concentrations were analysed (compare Figure 1, lower panel). No statistical differences between the molar activities of the three diuretics were obtained, identical dose-response relations could be calculated. During most experiments the reversibility of the inhibitory effect on the feedback sensor was also tested. Representative for all three diuretics, data of the piretanide study are given in the upper panel of Figure 1. The identity of SFP values before and after loop perfusion with the respective compounds verified the assumption of a completely reversible feedback inhibition.

Conclusions and Comments

The present study provides evidence for a reversible, concentration dependent inhibition of the tubuloglomerular feedback operation by various luminally applied loop diuretics. The high concentrations needed for effective feedback suppression suggest non-specific interactions between the diuretics and the feedback sensor of the macula densa. Since signal perception is postulated to depend on the rate of chloride ion transport by the macula densa segment [4], the present results of identical dose-response curves imply that all three diuretics should have identical activities in respect of the inhibition of electrolyte transport by this particular epithelium. A recent analysis of the intratubular effect of these diuretics on electrolyte transport in the thick ascending limb of Henle disclosed slight, albeit significant differences of their molar activities. The half maximal transport inhibition was achieved by lower doses of piretanide than of furosemide [5]. Thus functional differences between the electrolyte transport properties of the thick ascending limb and of the macula densa region must be assumed. In comparison with results of

\(^+\)Crystalline powders of all three diuretics have been generously supplied by Dr Lahn, Hoechst, Frankfurt, West Germany.
Figure 1. The upper panel of the figure compiles single measurements of SFP before, during and after perfusion of the loop with 50 nl/min physiological saline, containing logarithmically increasing concentrations of the diuretic compound piretanide [Ref.6]. The identity of the SFP values before and after the exposure of the macula densa to various concentrations of piretanide documents the reversibility of its effect on the feedback sensor. The lower panel displays the inhibition of the feedback response (given as ΔSFP on the ordinate) by each of the three diuretics tested. Excluding the results of the 10⁻⁸ M concentration group, the feedback inhibition became significantly more effective when the macula densa was exposed to progressively increasing diuretic concentrations. 10⁻⁴ M resulted in a complete inhibition of the feedback signal perception. Log-linear dose response curves were disclosed within the concentration range 10⁻⁸ to 10⁻⁴ M for each of the diuretics tested. No significant differences existed between the molar activities of furosemide, bumetanide and piretanide in respect of the feedback sensor.
Reference 5, it is apparent that the molar concentration of piretanide yielding a 60% inhibition of the feedback operation is higher than the concentration needed for the same degree of transport inhibition within the thick ascending limb of Henle, whereas these concentrations are essentially equal in respect of furosemide. Since the overall action of a diuretic is at least determined by both the effect on the feedback sensor as well as on the electrolyte transport by the epithelium, the cumulative dose response relation can be modified considerably. In the case of piretanide, this implies a flattening of the dose response curve, a conclusion which is in agreement with experimental results in the literature [6].

References

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