The Effects of Uraemic Compounds on Oxygen Consumption and Mechanical Activity of Isolated Guinea Pig Hearts

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Summary

Creatinine (5.6–22.6 mg/100 ml) and guanidinosuccinic acid (8.7–35.2 mg/100 ml) did not significantly affect mechanical function and oxygen consumption in isolated guinea pig hearts.

Urea (60–600 mg/100 ml) significantly reduced mechanical activity and caused marked increase of oxygen consumption, thus indicating impaired heart function in terms of diminished ratios of $\frac{dP}{dt}$.

Norepinephrine-induced increase of rates of left ventricular pressure rise/fall and of oxygen consumption was not altered by creatinine and guanidinosuccinic acid. However, urea pre-treatment did affect these parameters. Although indirect evidence of a predominant effect of urea on cell membrane has been shown, interference with myocardial metabolism is likely.

Introduction

Myocardial cell function appears to be altered in uraemia. Mechanical function of the heart has been reported to be decreased as well as increased (Del Greco et al, 1969; Goss et al, 1967; Henderson et al, 1971; Knowlan et al, 1960; Penpargkul & Scheuer, 1972; Raab, 1944). Furthermore histological changes and metabolic disturbances (Gormak & Popov, 1969; Penpargkul et al, 1975) in the uraemic heart have been described. The existence of a specific uraemic myocardial depressant substance has been suggested (Bailey et al, 1967).

Others, however, did not find evidence to support the existence of a characteristic uraemic myocardial defect and attributed myocardial changes to hypertensive
heart disease (Langendorf & Pirani, 1947). In general, discussion has centered around the question whether myocardial alterations found in uraemia are primarily due to accumulation of the retained products of protein breakdown such as urea, creatinine or guanidine or whether they are secondary to related factors such as hypertension, hypervolaemia, anaemia or electrolyte disorders.

The aim of this study was to assess the acute effects of urea, creatinine, guanidinosuccinic acid (GSA) and thereafter of norepinephrine (NE) on the rate of left ventricular pressure rise (dp/dt max), the rate of left ventricular pressure decrease (dp/dt min) and on oxygen consumption in isolated healthy guinea pig hearts.

MATERIALS AND METHODS

Guinea pigs of either sex, weighing 250–350 g were used in these experiments. After ether anaesthesia the hearts were rapidly isolated and perfused via the aorta at a constant flow of 10 ml/min by means of a peristaltic pump. The perfusion medium used was a modified tyrode solution (composition in mM/l: Na⁺ 149, K⁺ 5.4, Ca²⁺ 0.9, Mg²⁺ 1.05, Cl⁻ 145, H₂PO₄⁻ 0.42, HCO₃⁻ 11.9, glucose 11.0, Tris buffer 10.0, EDTA 0.05), maintained at 37°C and equilibrated with carbon (95% O₂-5% CO₂). Ascorbic acid (5 x 10⁻⁵ g/ml) was added as an antioxidant. Mechanical activity and oxygen consumption were determined simultaneously. The rates of left ventricular pressure rises (dp/dt max) and of pressure decreases (dp/dt min) of the electrically stimulated hearts (Grass S 5: square waves, 180/min, 5 m sec, 60 V) were measured using a Statham P 23 C transducer connected with a fluid-filled Latex balloon which was placed in the left ventricle. Oxygen consumption was measured by a modified polarographic method of Klaus and Krebs (1968).

After a 45 min equilibrium period perfusion with either urea, creatinine or GSA was started. Stepwise increase of concentrations followed as soon as steady state at each concentration level was reached. Then, on top of each cumulative dose response curve NE in two concentrations of 1 x 10⁻⁸ and 1 x 10⁻⁷ g/ml was added. Finally, after perfusion, the K⁺-concentration of the solution was measured by atomic absorption spectrophotometry. For statistical analysis Student’s t-test, either paired or unpaired, has been used as appropriate.

RESULTS

As shown in Figure 1, the increase of creatinine and GSA concentrations is accompanied by a slight but not significant decrease of dp/dt max and dp/dt min. Oxygen consumption of 256.2 ± 9.8 and 259.8 ± 3.7 (x ± SEM) µl/min/g under control conditions remained essentially the same after maximum concentration of either substance. With urea (Figure 2) there was a continuous fall of dp/dt max from a control value of 1101.7 ± 84.9 to 1002.8 ± 104.6 mmHg/sec (p < 0.05).
Figure 1. Mean ± SEM of dp/dt max, dp/dt min and QO₂ with creatinine and GSA in the dose range 0.5–2.0 x 10⁻³ M
Figure 2. Dose response curves of urea (10–100 mM)
The rate of relaxation showed a dose dependent and significant fall from 856.8 ± 84.1 (control) to 735.0 ± 48.1 mmHg/sec (at 100 mM concentration). Oxygen consumption was markedly increased from 269.0 ± 8.3 to 290.5 ± 8.0 µl/min/g even with the lowest concentration of 10 mM urea. Further increase of urea concentration did not cause significantly higher oxygen consumption.

Not surprisingly, the ratios of \( \frac{dp/dt}{QO_2} \) showed a similar and dose dependent

![Graph showing data with error bars and significance levels]

Figure 3. Effects of urea on ratios of \( \frac{dp/dt}{QO_2} \) max and \( \frac{dp/dt}{QO_2} \) min in mmHg/sec µl/min/g
Figure 4. Effects of urea (U), creatinine (Cr), and guanidinosuccinic acid (GSA) on norepinephrine (NE) induced alterations of mechanical function and oxygen consumption. Empty bar represents control (without pretreatment)
decrease with urea (Figure 3). However, after creatinine and GSA the ratios under control conditions were not altered significantly at any concentration used.

The effect of NE in concentrations of $1 \times 10^{-8}$ and $1 \times 10^{-7}$ g/ml is shown in Figure 4. Only urea dose response curves were followed by a significantly diminished increase of the three parameters measured. In contrast, creatinine and GSA pre-treatment did not alter the effects of NE. The increase of the ratios $\frac{dp/dt}{Q_O_2}$ (max, min), due to NE administration, remained unchanged after creatinine and GSA. However, urea reduced the effect of $1 \times 10^{-7}$ g/ml NE on $\frac{dp/dt}{Q_O_2}$ significantly.

The potassium concentration of the tyrode solution was not found to be altered during perfusion with either of the substances used. Osmolarity never exceeded the maximum value of 432 mOsm/l during perfusion with 100 mM urea.

**DISCUSSION**

The concentrations of the uraemic compounds employed were in the range common in uraemia. A GSA-induced decrease of cardiac contractility, found in healthy dogs by Aquatella et al (1975), could not be confirmed in isolated guinea pig hearts. Maximum creatinine concentrations exerted only a slight but not significant cardiodepressive effect. Urea, however, produced a marked cardiodepressant effect, thus confirming the findings of Scheuer and Stezoski (1973). This effect does not appear to be due to an increase in the osmolarity of the perfusion medium for two reasons: firstly, equiosmolar concentrations of sucrose did not produce a significant change of heart function as controlled in our apparatus. Secondly, urea in concentrations up to 400 mOsm/l has been observed to enhance contractile performance of ventricular myocardium (Koch-Weser, 1963), whereas a rise of osmolarity to 500 mOsm or more decreased contractility in atrial muscle (Schmidt et al, 1972). Breakdown products such as ammonium and cyanate accumulate when high concentrations of urea were permitted to remain in solution for long periods (Marier & Rose, 1964). Development of such breakdown products has been excluded in our study by making the solution fresh each day.

The mode of action of urea-induced myocardial depression remains uncertain. The rate of left ventricular pressure rise was found to be lowered to a lesser extent than the rate of relaxation. This differentiation does not allow us to interpret this as an intracellular effect of urea on Ca$^{++}$-elimination rather than on Ca$^{++}$-increase within the myocardial cell. The tendency for potassium ions to diffuse towards extracellular fluid in the isolated frog heart has been suggested to be an effect of urea permeability (Pankow & Pohle, 1968).

In our study urea did not alter potassium ion concentration in the perfusion medium. However, reversal of the negative inotropic effect of urea by application of NE would be in accordance with the concept that the cell membrane is the
site of action of urea. Catecholamines produce an increase of cell permeability to cations with preference for calcium ions, allowing these ions to pass into the myocardial cell (Reuter & Wollert, 1967). The steep increase of oxygen consumption in the low concentration range with virtually no further concentration-dependent increase may indicate that urea has a more pronounced effect on myocardial metabolism at low concentrations than at high concentrations, when permeability is affected predominantly. In this study the hearts were exposed to uraemic compounds for a short period. Chronic exposure, as might be seen in chronic uraemia, is an important variable to be considered before translating these data to clinical states.

Acknowledgment

This study was supported by a grant from the Landesamt für Wissenschaft und Forschung, Nordrhein-Westfalen.

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Open Discussion

PARSONS (London) We have been interested in uraemic cardiomyopathy for some time. Some people believe that it does not exist, others like yourself are beginning to wonder. Now the problem here is the osmolarity changes. You said that the only way you checked this was to use sucrose as a contrast substance, but the permeability of heart muscle to sucrose and urea is probably quite different. I do not know whether you measured the weight of your hearts at the end of the experiment but I imagine the uraemic hearts would be very much more oedematous than the sucrose hearts that had been perfused at the same osmolarity. Did you weigh them?

KERSTING We weighed the heart muscle, but we could not find any significant difference after perfusion with sucrose in equiosmolar concentration and after perfusion with urea. We cannot exclude the possibility that sucrose is affecting permeability in another way than urea.