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Effects of different doses of acetylsalicylic acid on renal oxygen consumption

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The aim of the present study was to examine the acute effect of acetylsalicylic acid (ASA) on renal oxygen consumption (Q_{O_2}) and renal sodium excretion ($U_{Na}V$) in anaesthetized dogs. With plasma salicylic acid (SA) concentrations ranging from 200–400 μg/ml, Q_{O_2} increased 36% ($P<0.05$) in spite of a 16% decrease in renal blood flow. At plasma SA concentrations of 80–200 μg/ml Q_{O_2} was significantly increased 45 min after the onset of ASA infusion. $U_{Na}V$ decreased from 97·7 to 21·5 μmol/min ($P<0.05$). Glomerular filtration rate and absolute tubular reabsorption of sodium (R_{Na}) was unchanged. The ratio R_{Na}/Q_{O_2} decreased from 31·0 to 21·3 ($P<0.05$). Renal lactate uptake increased. The results are most consistent with an uncoupling effect of ASA on oxidative phosphorylation in the kidney.

Key-words: acetylsalicylic acid, oxidative phosphorylation, renal blood flow, renal oxygen consumption, tubular reabsorption of sodium

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In a previous study [2] a significant decrease in renal sodium excretion and renal tubular PAH transport were found after intravenous administration of acetylsalicylic acid (ASA) to dogs. The main purpose of the present study was to investigate the effect of increasing intravenous ASA doses on renal oxygen consumption (Q_{O_2}) in anaesthetized dogs. Salicylates, like dinitrophenol, uncouple oxidative phosphorylation *in vitro* [5]. It has been shown that dinitrophenol increases renal oxygen consumption in dogs [16], but whether salicylates have the same effect *in vivo* is unknown.

It is believed that the bulk of sodium reabsorption in the kidney requires energy from oxidative phosphorylation [12]. If ASA increases the renal Q_{O_2}, the linear correlation between Q_{O_2} and active tubular reabsorption of sodium (R_{Na}) [10, 13] could be altered. The ratio R_{Na}/Q_{O_2} was therefore estimated at therapeutic and toxic plasma salicylic acid (SA) levels.

METHODS

Five adult mongrel dogs of both sexes weighing from 18–24 kg (mean, 20·8 kg) were used. The animals were fasted for 12 h but had free access to water before the experiment.

The surgical procedure was performed in

pentobarbital anaesthesia as described previously [2]. Artificial ventilation through an endotracheal tube was given by a Bennett respirator. The intravenous fluid infusions with 5% mannitol and 0·7% NaCl, priming and sustaining solutions of creatinine (Cr) and para-aminohippuric acid (PAH), urine sampling and clearance periods have also been described previously [2].

Blood from aorta, the renal vein and inferior caval vein was drawn every 15 min for analyses of PAH, blood gases, pyruvate and lactate. The samples were taken without suction in the middle of the clearance periods, after preceding aspiration of 6–7 ml blood. Arterial and venous blood samples were drawn within 1 min of each other. Blood samples were collected anaerobically for measurements of pH, P_{O_2}, P_{CO_2} and spectrophotometric Hb_{O_2} determination. Blood for lactate and pyruvate determinaations were placed directly into previously tared test tubes containing the appropriate volumes of cold trichloracetic acid (10% w/v) for instant precipitation of enzyme proteins. The exact volume of blood in the tubes were calculated from the weight of collected blood. Blood for other chemical analyses were drawn into heparinized plastic tubes, immediately centrifuged and the plasma removed.

Acetylsalicylic acid (ASA) (0·9 g lysine-acetylsalicylate, 0·1 g glycine, Aspegic®, Egic Laboratories) was given intravenously as injections, followed by infusions at increasing dose levels (7–200 mg/kg) and the treatment periods (I–IV) were determined from the plasma salicylic acid (SA) level: I, 20–80 μg/ml; II, 80–200 μg/ml; III, 200–400 μg/ml; and IV, >400 μg/ml. The values of each treatment period and the control period (C) were each the the mean of 3–4 clearance periods of 15 min duration.

ANALYTICAL METHODS

The analytical methods used for determination of sodium, creatinine, PAH, haematocrit (Hct), total and free salicylate, pH, P_{O_2} and P_{CO_2} have been described previously [2].

Blood gases were measured by duplicate analyses immediately after collection. The oxygen saturation of haemoglobin (HbO_2) was then calculated from P_{O_2} and P_{CO_2} by the Astrup method (Radiometer P_{O_2} electrode, type E 5046), using a standard dissociation curve for dog blood. HbO_2 was measured in duplicate spectrophotometrically by a Radiometer oxygen saturation meter, Type OSM 1, (Copenhagen, Denmark). The coefficient of variance for the chemical analyses of the O_2 content in duplicate was less than 1% (0·02–0·06 vol%) by either method. The mean HbO_2 values reported in this communication were taken from data obtained on the same sample using both methods.

Lactate and pyruvate concentration in blood from aorta, v. cava and v. renalis were determined in duplicate by standard reagent kits (Boehringer, Mannheim, Germany) according to the manufacturer's instructions. The optical density was read at 366 nm on a Beckman spectrophotometer. The coefficient of variance for the chemical lactate analyses in duplicate was 0·75%.

Creatinine clearance (C_{cr}) was used as index for glomerular filtration rate (GFR). Renal extraction of PAH (E_{PAH}) was calculated from the formula: $E_{PAH} = (RA_{PAH} - RV_{PAH})/RA_{PAH}$ where RA_{PAH} and RV_{PAH} were the concentrations of PAH in the renal artery and renal vein. True renal plasma flow (TRPF) = C_{PAH}/E_{PAH}. Renal blood flow (RBF) = TRPF/(1 − Hct). Urinary sodium excretion ($U_{Na}V$) was expressed as μmol/min. Filtered load of sodium (F_{Na}) = $C_{cr} \times P_{Na} \times 0{\cdot}95$ (0·95 = Gibbs Donnan factor). Absolute tubular reabsorption of sodium (R_{Na}) = $F_{Na} - U_{Na}V$. Fractional reabsorption of sodium ($R_{Na}\%$) = $R_{Na} \times 100/F_{Na}$.

Renal oxygen consumption (Q_{O_2}) was calculated using the formula of Wolf [22]:

$$Q_{O_2}\ \text{ml/min} = RBF \times RA_{O_2} - (RBF - U_V) \times RV_{O_2}.$$

$$Q_{O_2}\ \mu\text{mol/min} = \frac{Q_{O_2}\ (\text{ml/min}) \times 100}{22{\cdot}4}$$

Renal artery (RA_{O_2}) and renal vein oxygen concentration (RV_{O_2}) were calculated from the oxygen saturation of haemoglobin (= Hct × 0·34) in arterial and venous blood using 1·36 as factor for oxygen combining capacity. Correction for physically dissolved oxygen was not performed. The coefficient of variance for the renal Q_{O_2} measurements (sum of chemical analysis errors and errors on renal arteriovenous blood sampling and renal flow measurements) was not estimated, but has been calculated to

TABLE I. Effect of different acetylsalicylic acid (ASA) doses on renal oxygen consumption, renal sodium transport and renal blood flow

Parameter	Experimental periods				
	Control	I	II	III	IV
(SA) (μg/ml)	0	29·0±4·9	144·0±3·6	289·0±30·0	512·0±41·0
HbO_2 (%) aorta	93·7±1·4	91·4±2·4	92·8±2·8	91·8±2·0	93·3±2·2
HbO_2 (%) v. renalis	81·3±2·8	76·5±3·2	73·5±3·0*	70·4±3·1*	70·0±3·1*
HbO_2 (%) v. cava	70·2±4·4	57·7±7·0*	48·9±6·3*	39·0±6·5*	27·8±5·6*
Q_{O_2} (μ mol/min)	164·7±14·6	181·8±13·7	217·1±9·4*	224·6±8·3*	233·5±23·0*
$U_{Na}V$ (μ mol/min)	97·7±27·8	45·4±16·3*	27·2±4·7*	21·5±7·3*	56·6±27·3
R_{Na} (%)	98·07±0·38	99·03±0·29*	99·50±0·10*	99·54±0·19*	98·88±0·42
R_{Na} (μ mol/min)	5105±436	4688±272	4904±247	4826±303	4976±406
$R_{Na}/Q_{O_2}-1$	31·4±0·9	25·8±1·3*	22·6±1·9*	21·5±0·5*	21·3±1·6*
RBF (ml/min)	191·6±12·5	179·1±10·8	174·1±12·0	160·6±16·4*	151·4±19·4*
C_{cr} (ml/min)	39·1±3·0	35·7±1·9	37·2±1·6	36·6±2·2	37·9±2·8

For abbreviations, experimental conditions and periods, see methods. The results are expressed as mean values± SEM for five dogs. * Different from control ($P<0·05$).

13% [4] in another study where comparable methods were used.

R_{Na}/Q_{O_2} = ratio between absolute tubular reabsorption of sodium (μmol/min) and total oxygen consumption (μmol/min) of the kidney. Renal consumption of lactate ($Q_{lactate}$) = $(RA_{lactate}-RV_{lactate})\times RBF$. Correction for urinary lactate excretion was not performed. 'Excess lactate' in arterial blood was calculated according to Huckabee [11].

The mean and standard error of the mean (SEM) were calculated and statistical comparisons were carried out according to Wilcoxon's method for paired differences. The results are given in the form of mean ± SEM unless otherwise stated.

RESULTS

Table I shows the effect of five experiments with increasing ASA doses on arterial and venous oxygen saturation, renal oxygen consumption, sodium reabsorption and renal blood flow. A progressive decline in $U_{Na}V$ was observed, from 97·7 ± 27·8 in the control period to 21·5 ± 7·3 μmol/min ($P<0·05$) in the treatment period III (SA, 200–400 μg/ml). Fractional sodium reabsorption (R_{Na}%) increased significantly and in inverse ratio to $U_{Na}V$. As GFR was unaltered, 39·1 ± 3·0 ml/min in the control period and 37·9 ± 2·8 ml/min in period IV, absolute sodium reabsorption (R_{Na}) showed only small alterations, dependent on small fluctuations in GFR and plasma sodium concentrations.

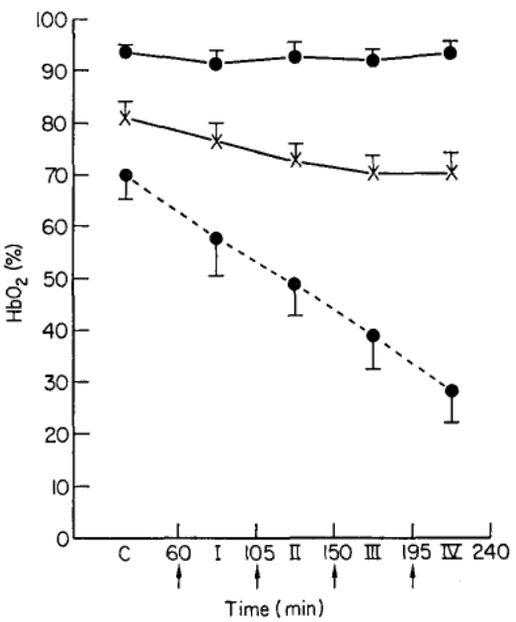

FIG. 1. Percent oxygen saturation of haemoglobin (Hb_{O_2}) in aorta (●), v. renalis (×) and v. cava (dotted line) in the control period (C) and in the 4 ASA treatment periods referred to in methods. The results are expressed as mean values ± SEM for five dogs. Roman numerals I-IV indicate the treatment periods at increasing ASA doses, each of them being the mean of 3-4 clearance periods.

Renal blood flow decreased from 191·5 ± 12·5 (C) to 151·4 ± 19·4 (IV) ml/min (21%) ($P<0·05$). True plasma flow decreased from 127·2 (C) to 104·6 ml/min (IV). Oxygen saturation of arterial haemoglobin was unaltered and within normal limits throughout the experiment. The oxygen saturation of haemoglobin in the caval

TABLE II. The effect of increasing acetylsalicylic acid (ASA) doses on urinary sodium excretion ($U_{Na}V$), creatinine clearance (C_{cr}), tubular reabsorption of sodium (R_{Na}), renal oxygen consumption (Q_{O_2}) and R_{Na}/Q_{O_2} in a dog

	Clearance-period	Time (min)	$U_{Na}V$ (μmol/min)	C_{cr} (ml/min)	R_{Na} (μmol/min)	Q_{O_2} (μmol/min)	$\frac{R_{Na}}{Q_{O_2}}$
	1	−45–30	105·6	36·9	4912	186·2	26·4
C	2	−30–15	131·8	35·7	4688	162·5	28·9
	3	−15–0	144·5	36·4	4769	177·2	26·9
⟶							
	4	0–15	115·8	36·5	4812	175·5	27·4
I	5	15–30	62·8	34·1	4575		
	6	30–45	35·3	34·0	4589	184·4	24·9
⟶							
	7	45–60	24·3	35·0	4571	191·5	23·9
II	8	60–75	15·8	35·6	4826		
	9	75–90	13·0	34·9	4787	208·5	22·9
⟶							
	10	90–105	14·6	34·9	4880	271·0	18·0
III	11	105–120	14·2	37·2	5082		
	12	120–135	14·2	37·9	5178	274·6	18·9

The arrows indicate start infusion of ASA at increasing dose levels (I–III). Each treatment period and the control period (C) consist of three urine sampling periods of 15 min duration (1–12), see Methods.

vein decreased in all dogs, from 70·2 ± 4·4 to 27·8 ± 5·6% ($P < 0·05$). Consequently, systemic arteriovenous oxygen difference increased more than twofold, from 3·65 to 9·60 ml/100 ml. Total body oxygen consumption and cardiac output were not measured. The oxygen saturation of haemoglobin in the renal vein decreased from 81·3 ± 2·8 to 70·4 ± 3·1% ($P < 0·05$) (Fig. 1).

Renal arteriovenous oxygen difference showed a small and inconsistent increase during the first treatment period, followed by a further and pronounced increase in all dogs at higher levels of ASA, from 1·93 (C) to 3·42 ml/100 ml (IV), ($P < 0·05$). In spite of a 21% decrease in renal blood flow (IV) ($P < 0·05$), renal oxygen consumption increased gradually, from 3·7 ml/min (164·7 μmol/min) (C) to 5·2 ml/min (233·5 μmol/min) (IV). The alterations induced were significant ($P < 0·05$) in periods II, III and IV. The effect of ASA on Q_{O_2} started gradually and was not significant at the lowest plasma SA levels. When plasma SA was increased to 80–200 μg/ml (45–90 min after start ASA infusion), Q_{O_2} increased in all five dogs.

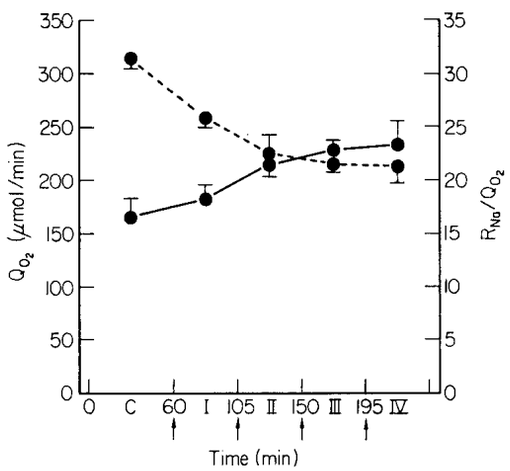

FIG. 2. Renal oxygen consumption (Q_{O_2}) (left ordinate) (bold line) and ratio of renal tubular sodium reabsorption/renal oxygen consumption (R_{Na}/Q_{O_2}) (right ordinate) (dotted line) in the control period (C) and in the four treatment periods with ASA (I–IV). The results are expressed as mean values ± SEM for five dogs.

The effect of urinary sodium excretion was seen after the lowest ASA dose, 15–30 min after the treatment was started, in some dogs even within the first 15 min. Table II gives an example of such an experiment (treatment periods I–III) where $U_{Na}V$ decreased within the first 15 min period, whilst an increase in renal oxygen consumption was seen later (periods II–III), after higher ASA doses. As absolute sodium reabsorption (R_{Na}) did not change, the experimental R_{Na}/Q_{O_2} ratio declined from 31·0 ± 0·9 (C) to 21·3 ± 1·6 (IV) ($P < 0·05$) (Fig. 2).

Table III summarizes the effect of different ASA doses on renal arterial and venous lactate concentrations and renal lactate uptake. Arterial lactate concentrations increased from 0·438 ± 0·148 to 1·056 ± 0·299 μmol/ml, and the venous

TABLE III. The effect of different acetylsalicylic acid (ASA) doses on renal arterial and venous lactate concentrations and renal lactate consumption

Period	Lactate μmol/ml		$Q_{lactate}$ (μmol/min)
	Aorta	v. renalis	
C	0·438±0·148	0·378±0·141	11·5±3·4
I	0·428±0·131	0·290±0·037	25·0±11·7
II	0·501±0·137	0·291±0·047	36·9±12·5
III	0·600±0·132	0·458±0·057	23·2±10·2
IV	1·056±0·229	0·524±0·046	81·3±19·3

For experimental conditions and periods, see Methods. The results are expressed as mean values± SEM for five dogs.

concentrations from 0·378±0·141 to 0·524± 0·046 μmol/ml. Arterial pyruvate concentration was unchanged, 0·0519±0·017 (C) and 0·0598± 0·018 μmol/ml (IV), and 'excess lactate' of arterial blood 0·56 μmol/ml (IV). Renal lactate uptake ($Q_{lactate}$) increased from 11·5±3·4 to 81·3±19·3 μmol/min.

Arterial pH was stable and within normal limits (7·29±0·02 (C) and 7·30±0·03 (IV)). Arterial P_{CO_2} was unchanged from C (36·6± 3·0) to III (35·8±4·1) but fell to 31·4±3·9 mmHg at the highest dose levels. Plasma electrolytes (Na, K, Cl) showed no consistent changes. Mean blood pressure decreased from 122·9± 4·2 (C) to 116·6±5·4 mmHg (IV).

DISCUSSION

In the present study i.v. administration of ASA increased renal oxygen consumption in all of five dogs exhibiting plasma SA levels from 80–200 μg/ml. At toxic ASA dose levels (plasma concentrations of SA higher than 400 μg/ml) the increase in Q_{O_2} was 42%.

The method employed for Q_{O_2} determination includes several sources of error, but the ASA changes induced in this study are much greater than the coefficient of variation for the Q_{O_2} measurements [4]. From the present results one is therefore apt to conclude that ASA, like dinitrophenol, increases renal oxygen consumption *in vivo*.

Renal blood flow decreased significantly in this study. Other workers have found that renal oxygen consumption either is uninfluenced [7, 13] or positively correlated [4, 14] with renal blood flow. Thus, the increase in Q_{O_2} in the present investigation can not be explained by alterations in renal blood flow.

Systemic arteriovenous oxygen difference was increased by 100% after i.v. ASA doses resulting in plasma SA concentrations from 200–400 μg/ml. An increase in systemic oxygen consumption of 60–80% has been reported after i.v. administration of sodium salicylate (100 mg/kg) in anaesthetized dogs [18]. Since total oxygen consumption was increased by ASA it is not surprising that renal Q_{O_2} increased in our study. The increase in systemic oxygen consumption and renal Q_{O_2} was significant at serum SA levels corresponding to low 'anti-inflammatoric' doses of ASA in man (plasma SA, 80–200 μg/ml).

GFR, measured as exogenous creatinine clearance, was unchanged in this study. An underestimation of endogenous creatinine clearance by salicylates has been reported [6]. We have previously recorded that the administration of comparable ASA doses as used in the current investigation, will not alter endogenous creatinine clearance in normal men [1], nor change inulin clearance in dogs [3]. Exogenous creatinine clearance appears therefore to be a valid parameter for GFR in the present study.

Urinary sodium excretion was significantly reduced at all therapeutic dose levels of ASA used. Consequently, fractional sodium reabsorption increased. The ratio sodium reabsorbed/renal oxygen consumed decreased from 31·4 to 21·3. This is similar to the effect observed in anaesthetized dogs given 12–16 mg dinitrophenol/kg i.v. [16]. Since ASA seems to decrease sodium excretion at lower doses than those needed to increase renal oxygen consumption, these two effects of ASA appear to be independent. It has also been shown that SA, which exerts more pronounced effects than ASA on oxidative phosphorylation *in vitro* [5, 19], increases urinary sodium excretion in dogs [17]. Sodium excretion is reported unchanged [9, 21] or decreased after dinitrophenol [16].

Renal sodium transport is the process which requires most energy in the kidney and under many conditions there is a stoichiometric relationship between R_{Na} and Q_{O_2} which has been found to vary from 24 to 28 Eq Na per mole of oxygen consumed in anaesthetized dogs [7, 10, 13]. An increased oxygen consumption is a theoretically expected consequence of an

ineffective oxidative phosphorylation. Uncoupling drugs should increase the rate of oxidation of endogenous and exogenous substrates to compensate for the relative inefficiency of the phosphorylating mechanisms. During treatment with ASA the energy requirement for renal sodium transport seems to be increased and consequently, the ratio R_{Na}/Q_{O_2} decreased. It is also reasonable to assume that the oxygen requirement for other renal metabolic processes (basal oxygen consumption) is increased by ASA.

If salicylates really uncouple oxidative phosphorylation in the kidney *in vivo*, then the renal 'energy charge' (defined as the ATP/ADP ratio) should fall. Other workers have indeed found a reduction in renal cortical ATP/ADP ratio after intrarenal infusion of sodium salicylate [15] and dinitrophenol [12, 21] in dogs. Since mitochondrial ATP is the major source of energy for renal sodium transport, it is somewhat surprising that sodium excretion decreased in our study. It is conceivable that the possible inhibition of sodium reabsorption induced by an uncoupling effect of ASA is counteracted by the haemodynamic effects of ASA in the kidney. ASA reduces renal blood flow, probably by an inhibitory effect on prostaglandin synthesis [20].

Arterial lactate concentration increased after toxic ASA doses without concomitant change in arterial pyruvate concentration (increased 'excess lactate'). This increase is probably due to alterations in cellular oxidation, either due to hypoxia or due to an uncoupling effect of ASA. Hyperventilation has been shown to increase both arterial lactate and pyruvate concentrations in dogs [11]. In this study arterial pCO_2 was slightly reduced at the highest plasma SA levels achieved following the i.v. administration of ASA. However, as pyruvic acid did not accumulate, hyperventilation is probably not the reason for the lactate accumulation.

Renal lactate uptake increased in parallel with the arterial lactate concentration. This uptake has been shown to be the function of at least two main factors: arterial lactate concentration and renal tubular sodium reabsorption [8]. Since we did not measure other substrates, we can not link sodium reabsorption to the observed uptake of lactate. Our data indicate that there was no output of lactate from the kidney and, consequently, no ischaemic changes in this organ despite the rise in renal oxygen consumption.

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