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The effect of acetylsalicylic acid on renal function in the Pekin duck

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1 The acute effects of intravenously administered lysine-acetylsalicylic acid (ASA) on renal function in the Pekin duck have been studied with special reference to possible interactions with the antidiuretic hormone, arginine vasotocin (AVT), in the control of renal water and solute output.

2 ASA produces an immediate increase in urine flow rate which is dose-related in the range 25 to 100 mg kg^{-1} and is associated with a slight reduction in urine osmolality, but an overall increase in renal osmolal excretion affecting Na^+, Cl^- and K^+ to approximately equal extents.

3 The effects, which are similar in both saltwater and freshwater adapted ducks infused with hyposmotic saline or glucose solution, can also be produced by similar doses of sodium salicylate (SA).

4 The mechanism of action is probably not related to inhibition of prostaglandin synthetases.

5 There is no change in the glomerular filtration rate or peripheral blood pressure following the ASA injection.

6 There is no change in the circulating level of AVT; however, preliminary studies do not exclude the possibility of a partial antagonism of salicylate to AVT at the renal level.

Introduction

Pekin ducks submitted to combined salt and water loading were observed to exhibit widely different states of diuresis with similar circulating levels of antidiuretic hormone, arginine vasotocin (AVT). According to unpublished findings of Simon *et al.* (1982), ducks with plasma AVT levels of 12–13 pg ml^{-1} and plasma osmolalities of 301 to 303 mosm kg^{-1} were strongly antidiuretic when infused with 0.4 ml min^{-1} of 1000 mosm kg^{-1} NaCl solution, but excreted 1 ml min^{-1} of hypotonic urine when submitted to infusion of 1.6 ml min^{-1} of 250 mosm kg^{-1} NaCl solution, the differences in osmotic stress being compensated by the contributions of the salt secreting supraorbital glands (Simon, 1982). This observation prompted the thought that perhaps something other than AVT was involved in the control of this diuresis. The postulated interference of prostaglandins with the action of antidiuretic hormone (ADH) as suggested by Anderson *et al.* (1975), Berl *et al.* (1977), Fejes-Tóth *et al.* (1977), Bisordi *et al.* (1980), Schlondorff *et al.* (1981) and others indicated a possible candidate for investigation. We therefore investigated the effect of acetylsalicylic acid (ASA), a prostaglandin synthetase inhibitor (Vane, 1971), upon steady state diuresis induced by the constant infusion of hyposmotic saline or glucose in conscious Pekin ducks adapted either to saltwater or to freshwater as drinking fluid.

We also examined the effect of sodium salicylate (SA) under the same conditions in order to characterize the effect of another salicylate in birds. This was done because in mammals the reduction in the excretion of both water and solutes by the kidneys ascribed to ASA (Gardier *et al.*, 1962; Berg & Bergan, 1976; Berl *et al.*, 1977) is not consistently observed (Berg & Bergan, 1976) and SA is reported to increase these two parameters (Ramsay & Elliot, 1967; Quintanilla & Kessler, 1973).

There is currently no information available concerning the effects of prostaglandin synthetase inhibitors in general or salicylates in particular, upon renal function in birds. Proceeding from the idea of differences in the sensitivity of renal excretion to ADH in birds, depending on the type of salt and water loading, it was the main purpose of this study to investigate the effects of an intravenous preparation

of ASA (Aspisol) on renal water and solute excretion in Pekin ducks, focussing particular attention upon possible interactions between ASA and AVT. We have also tested other prostaglandin synthetase inhibitors (indomethacin, meclophenamate) (Flower, 1974) and determined the effect of SA in an attempt to elucidate possible differences in the mechanisms of action.

Methods

Animals

The studies was carried out with adult male and female Pekin ducks, within a weight range of 2–3 kg, housed in flocks at room temperature of 23 ± 2°C with a natural day-night cycle. The animals were maintained on dry chicken food enriched with minerals and vitamins. One group of animals was given tap-water as drinking fluid and these ducks are subsequently referred to as 'water ducks'. The second group, subsequently referred to as 'salt ducks' were reared on a saline solution as the sole drinking fluid with increasing concentration, the final concentration being about 1.9% which corresponds to an osmolality of 600–620 mosm kg^{-1}. In these ducks the supraorbital glands become adapted to excrete NaCl as a highly concentrated solution of about 1000 mosm kg^{-1} at a rate of up to 0.8 mosm min^{-1}. The salt ducks were maintained permanently on 1.9% NaCl solution as drinking fluid.

Experimental procedure

The day before an experiment the animals were deprived of food, with free access to tap water and saline solution respectively, for drinking. The experiments were performed at a room temperature of 23 ± 2°C. On the morning of the experimental day, an animal was suspended in a cotton sling which prevented the animal from turning around but allowed free movements of the neck and feet. A flexible cannula was inserted into one wing vein for continuous intravenous (i.v.) administration of various solutions with an infusion pump. Blood samples were withdrawn from a second cannula which was placed in a leg vein and kept patent by a constant infusion of 0.1 ml min^{-1} of 0.9% saline. Insertion of the two cannulae was very quick and simple, causing the birds no observable pain or distress. In some cases, on the day before the experiment, the birds were anaesthetized (Halothane, N_2O, O_2) and a catheter placed into a brachial artery for the measurement, via a pressure transducer (Endevco), of mean arterial pressure, pulse pressure and heart rate.

Renal excretion

When an animal had been placed into the experimental stand, 1.6 ml min^{-1} of hyposmotic saline (250 mosm kg^{-1}) or of glucose solution (200 mosm kg^{-1}) was continuously infused to establish a steady state diuresis. A tube ending with a perforated bulb was inserted into the cloaca and kept in position by taping it to the tail feathers. The urine discharged into the cloaca was continuously withdrawn by suction into graduated cylinders and measured to an accuracy of ± 0.1 ml in 5–15 min intervals. Due to the fasting period the collected urine was practically free of faeces.

Salt gland secretion

In the salt ducks, part of the NaCl infused with 1.6 ml min of 250 mosm kg^{-1} saline is excreted by the supraorbital salt glands via the nares. The experimental animal had a silastic tube inserted into each nostril, secured to the bill by adhesive tape and connected to a permanent suction for removal of the salt gland fluid. The fluid was continuously withdrawn into pre-weighed vials which were exchanged every 10 min to determine the rate of secretion by weighing.

Measurements

The osmolalities of the urine, salt gland fluid and of blood plasma recovered from the blood samples by centrifugation were determined by the freezing point depression method with a Knauer Halbmikro-Osmometer or the vapour pressure method with a Wescor Osmometer. Na^+- and K^+-concentrations were determined by flame photometry (IL 543) and Cl^- by coulometric titration (Corning EEL). The AVT analyses were carried out on plasma from 1.5 ml blood samples withdrawn from the leg vein and immediately centrifuged at 1–2°C. The plasma was stored frozen at −20°C until assayed for AVT content within the next week by means of a radioimmunoassay (RIA) based on the high cross-reactivity of AVT with an AVP antiserum (for details see Gray & Simon, 1983). Renal glomerular filtration (GFR) was determined under continuous infusion of [^{14}C]-inulin, the concentrations in blood and urine samples being determined by beta-spectrophotometry. In the experiments in which arterial pressure was determined, pulse and/or the electrically integrated mean pressure were continuously recorded on a Brush-Gould 260 direct writer.

Salicylate administration and measurement

Solutions of acetylsalicylic acid (Aspisol, Bayer,

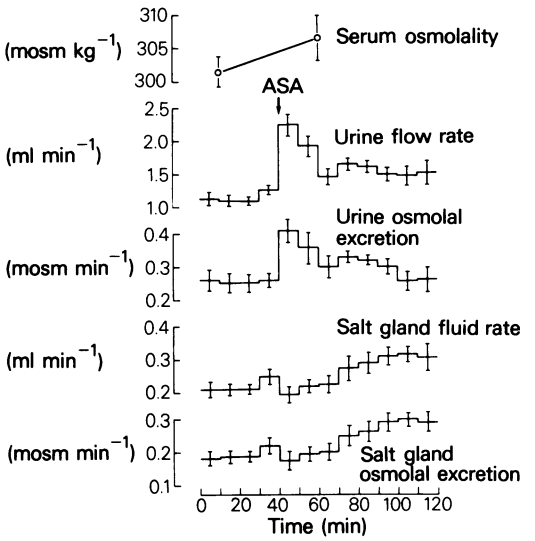

Figure 1 Effects of i.v. acetylsalicylic acid (ASA, 75 mg kg^{-1}) on blood osmolality, urine flow rate, urine osmolal excretion, salt gland secretion rate and osmolal excretion in 7 conscious salt ducks during i.v. infusion of 1.6 ml min^{-1} of 250 mosm kg^{-1} NaCl solution. Means with s.e. indicated by vertical lines.

Leverkusen) and sodium salicylate (Sigma, Muenchen) in water were prepared immediately before use and given as intravenous injections. The acidic nature of the solutions (pH 5.6) necessitated slow injection (1.5–2.5 ml given in 3 min) to avoid irritation of the animal. Total salicylic acid in plasma was determined fluorometrically by a modification of the alkaline hydrolysis method of Putter (1975) using 300 nm for activation and 406 nm for emission.

Further drugs used in some experiments were synthetic arginine vasotocin (Serva, Heidelberg), indomethacin (Sharp & Dohme Muenchen) and meclophenamate (Parke-Davis, Muenchen). In order to assess the role of endogenous arginine vasotocin (AVT), an AVT-antiserum raised in rabbits was injected intravenously in certain conditions of diuresis.

Statistical evaluation

In the text and the figures the results are expressed as means and standard errors of the mean (s.e.). The differences between the control data and those of the experimental conditions were tested for statistical significance with the paired *t* test. The levels of significance were evaluated by applying the two-tailed test, i.e. the levels given in the text refer to 2*P*.

Results

Effect of acetylsalicylic acid in salt ducks

When in a steady state of diuresis established by the continuous i.v. infusion of 1.6 ml min^{-1} of hyposmotic saline (250 mosm kg^{-1}) the salt glands accounted for about 50% of the salt elimination in the salt ducks and the urine was clearly hyposmotic

Table 1 Parameters of renal excretion in salt water adapted ducks receiving 1.6 ml min^{-1} of 250 mosm kg^{-1} NaCl solution as continuous i.v. infusion; effects of i.v. injections of 75 mg kg^{-1} acetylsalicylic acid (ASA)

	Number of experiments	*−20 to 0 min before ASA*	*0 to +20 min after ASA*
Urine flow rate (ml min^{-1})	8	1.19±0.05	2.10±0.10**
Urine osmolality (mosm kg^{-1})	8	211.40±9.58	182.54±11.63**
Osmolal excretion (mosm min^{-1})	8	0.251±0.015	0.384±0.034**
Free water clearance (ml min^{-1})	8	0.35±0.04	0.82±0.09**
Osmolal clearance (ml min^{-1})	8	0.84±0.06	1.28±0.11**
U_{Na} (mEq min^{-1})	6	0.088±0.006	0.144±0.010**
U_{Cl} (mEq min^{-1})	6	0.088±0.006	0.142±0.014**
U_{K} (mEq min^{-1})	6	0.006±0.001	0.010±0.002*
Na/K-quotient	6	16.4±1.7	15.2±1.5 NS

Means with s.e., ** $2P<0.01$; * $2P<0.05$.

(211 ± 10 mosm kg^{-1}). The animals responded to ASA given i.v. at a dose of 75 mg kg^{-1} with an immediate and highly significant increase in their renal water and solute output. Figure 1 shows that ASA produced a rapid increase in urine flow rate, rising from a mean control level of 1.15 ± 0.08 ml min^{-1} to a peak of 2.43 ± 0.11 ml min^{-1} ($2P < 0.0001$). The osmolality of the urine fell by 40 mosm kg^{-1}; however, the overall renal osmolal excretion was significantly increased by ASA, rising from 0.25 ± 0.02 mosm min^{-1} to a peak of 0.41 ± 0.04 mosm min^{-1} ($2P < 0.001$). The induced increased diuresis which lasted for about 30 min caused the osmolality of the plasma to rise significantly from 301.4 ± 2.2 to 306.6 ± 3.5 mosm kg^{-1} ($2P < 0.005$). As also shown by Figure 1, a delayed activation of the salt gland secretion, obviously due to the rise in plasma osmolality, followed the diuretic response. This indicates that there was no central effect of ASA on the common afferent or integrative system controlling the two osmoregulatory effectors, the salt glands and kidneys, and, hence, the effect of ASA on the kidney must be a direct one.

Table 1 shows that the polyuric response to ASA comprised increases of both, free water clearance and osmolal clearance, the latter being accomplished by approximately proportional rises of the urine electrolytes. In particular, the outputs of Na^+ and K^+ increased by similar fractions so that the Na/K quotient of the urine did not change significantly.

Effect of varying doses of acetylsalicylic acid

Figure 2 shows that the renal effect of ASA was dose-related. A small increase in urine flow rate and solute output was produced by an ASA dose of 25 mg kg^{-1}, a response which increased with the dose so that 100 mg kg^{-1} ASA produced almost a doubling of both parameters. The routinely employed dose of 75 mg kg^{-1} induced a clearly defined, reproducible response. The total plasma SA concentration associated with this dose was determined in representative blood samples taken 1 min after the end of ASA injection, and was found to be 690 ± 22 μg ml^{-1}.

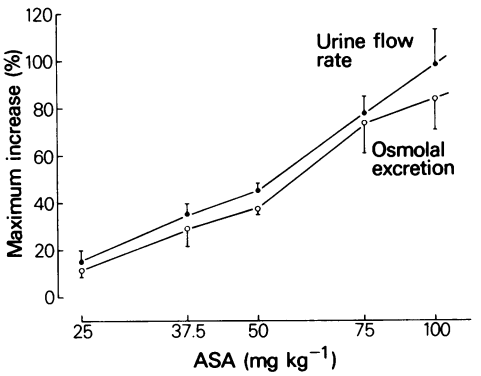

Figure 2 Dose-dependent effect of i.v. acetylsalicylic acid (ASA) on urine flow rate and urine osmolal excretion in conscious water ducks during i.v. infusion of 1.6 ml min^{-1} of 250 mosm kg^{-1} NaCl solution. Means with s.e. indicated by vertical lines.

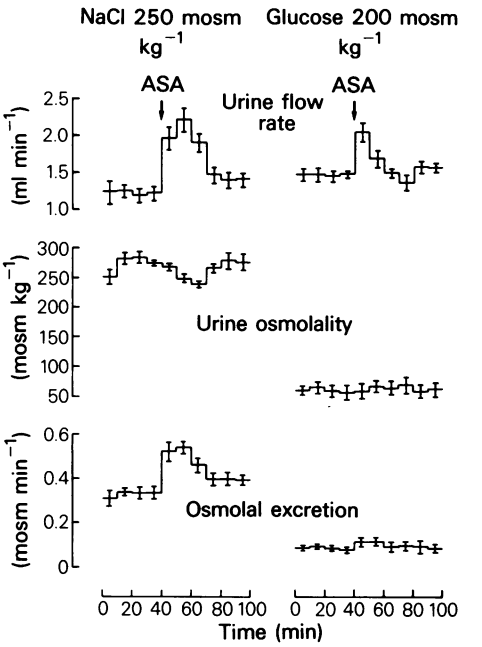

Figure 3 Effects of i.v. acetylsalicylic acid (ASA, 75 mg kg^{-1}) on urine flow rate, osmolality and osmolal excretion in 6 water ducks during i.v. infusion of either 1.6 ml min^{-1} of 250 mosm kg^{-1} NaCl solution or 1.6 ml min^{-1} of 200 mosm kg^{-1} glucose solution. Means with s.e. indicated by vertical lines.

Effect of acetylsalicylic acid in water ducks

ASA given i.v. at a dose of 75 mg kg^{-1} to water ducks infused with hyposmotic saline (250 mosm kg^{-1} NaCl) or glucose solution (200 mosm kg^{-1}) produced, in both groups, a pattern of response similar to that found in the salt ducks, although the urine osmolalities were somewhat higher in the saline loaded and much lower in the glucose loaded ducks. Figure 3 shows that the steady state diuresis of 1.21 ± 0.07 ml min^{-1} during saline infusion was increased by ASA to a peak of 2.18 ± 0.14 ml min^{-1} ($2P < 0.0001$). The osmolality of the urine was slightly reduced, but the overall osmolal excretion was increased from 0.32 ± 0.02 mosm min^{-1} in the

Table 2 Plasma osmolality and plasma arginine vasotocin (AVT) before and after 75 mg kg^{-1} acetylsalicylic acid (ASA) in ducks infused with 1.6 ml min^{-1} NaCl solution of 250 mosm kg^{-1}

	Before ASA		*20 min after ASA*	
	Osmolality (mosm kg^{-1})	*AVT* (pg ml^{-1})	*Osmolality* (mosm kg^{-1})	*AVT* (pg ml^{-1})
Salt ducks $n=7$	301.43 ±2.20	11.29 ±1.28	306.57*** ±3.51	11.06NS ±1.12
Water ducks $n=6$	299.33 ±3.81	10.14 ±2.34	303.17*** ±3.38	8.78NS ±1.49

Means with s.e.; *** $2P<0.005$, ** $2P<0.01$

control period to a peak of 0.57 ± 0.03 mosm min^{-1} immediately after the ASA administration ($2P<0.0001$). The use of the continuous infusion of glucose solution did not alter the response to ASA, although the duration of its effect tended to be shorter than with saline. The urine flow rate was increased from the control of 1.49 ± 0.06 ml min^{-1} to a maximum of 2.07 ± 0.13 ml min^{-1} ($2P<0.002$). There was no further decrease of urine osmolality. Accordingly, osmolal output increased from 0.09 ± 0.01 to 0.12 ± 0.02 mosm min^{-1} ($2P<0.01$).

Acetylsalicylic acid and circulating arginine vasotocin

Table 2 shows that in both salt and water ducks infused with 250 mosm kg^{-1} NaCl solution at 1.6 ml min^{-1}, there was no change in the plasma levels of AVT measured before and after an ASA dose of 75 mg kg^{-1}. Both groups of animals had settled down to similar plasma osmolalities around 300 mosm kg^{-1} and to similar plasma AVT concentrations of about 10-11 pg ml^{-1}. The plasma osmolalities had risen by about 5 mosm kg^{-1} 20 min after the ASA infusions, due to activation of diuresis, but changes of plasma AVT could not be confirmed statistically.

Another indication that ASA does not produce increased diuresis by an inhibition of the central release of AVT is given in Figure 4. Birds receiving a continuous i.v. infusion of 250 mosm kg^{-1} saline containing AVT at a concentration that maintained its plasma level at about 10 pg ml^{-1} responded to ASA in the same typical way as without external AVT maintenance.

Effect of sodium salicylate

Water ducks in a steady state diuresis produced by the infusion of 250 mosm kg^{-1} NaCl solution at 1.6 ml min^{-1}, responded to i.v. sodium salicylate with increased renal water and solute output in a dose-dependent manner. Figure 5 shows that the lowest effective dose was 12.5 mg kg^{-1} and the response linear up to a dose of 100 mg kg^{-1}.

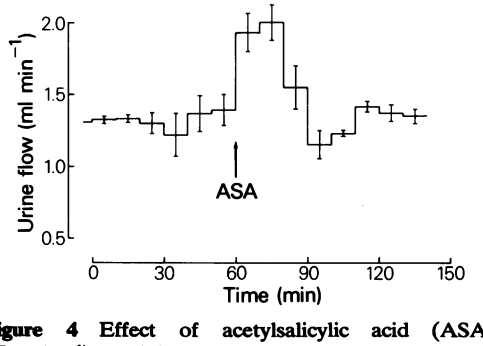

Figure 4 Effect of acetylsalicylic acid (ASA, 75 mg kg^{-1}) administered to 4 water ducks during i.v. infusion of 1.6 ml min^{-1} of 250 mosm kg^{-1} NaCl solution to which arginine vasotocin (AVT) was added to maintain AVT plasma concentration at a level of about 10 pg ml^{-1}. Means with s.e. indicated by vertical lines.

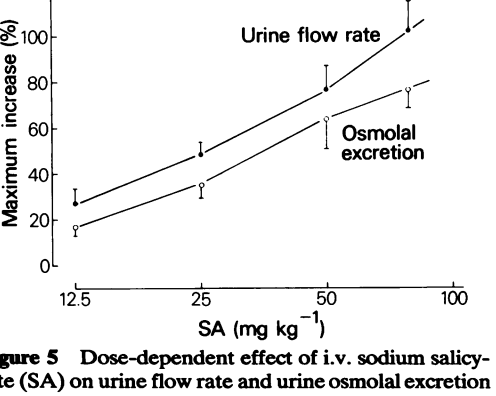

Figure 5 Dose-dependent effect of i.v. sodium salicylate (SA) on urine flow rate and urine osmolal excretion in conscious water ducks during i.v. infusion of 1.6 ml min^{-1} of 250 mosm kg^{-1} NaCl solution. Means with s.e. indicated by vertical lines.

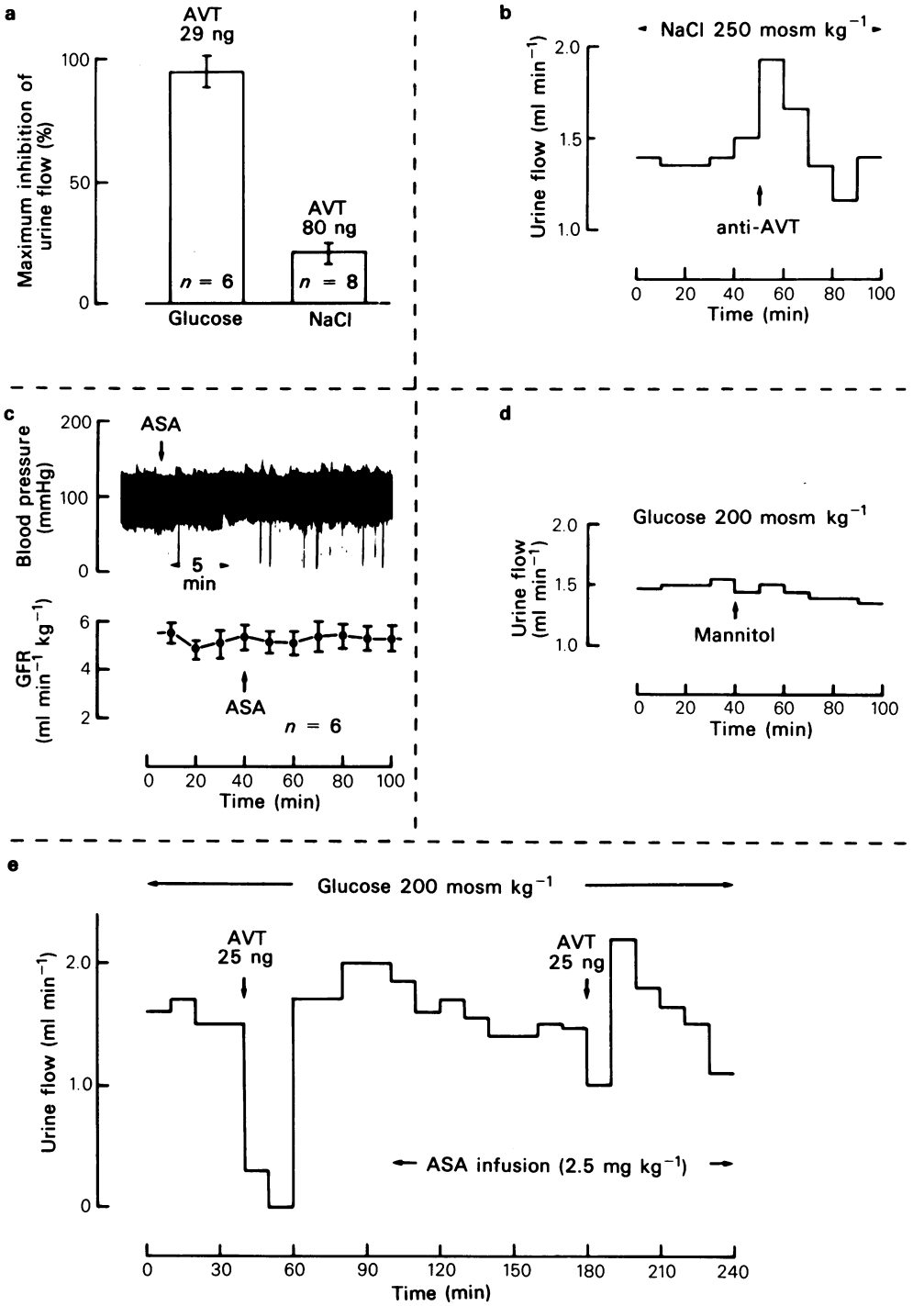

Figure 6 Results of preliminary studies relating to sensitivity changes of arginine vasotocin (AVT, a and b) and to possible mechanisms of acetylsalicylic acid (ASA) action (c to e). (a) Comparison of the antidiuretic responses to i.v. injected AVT in water ducks with comparable urine flow rates induced by i.v. infusion of either 1.6 ml min^{-1} of 200 mosm kg^{-1} glucose solution (left column) or 1.6 ml min^{-1} of 250 mosm kg^{-1} NaCl solution (right column). Means with s.e. indicated by vertical lines. (b) Single experiment in a water duck made diuretic by i.v. infusion of 1.6 ml min^{-1} of 250 mosm kg^{-1} NaCl solution: effect of i.v. injection of 1.5 ml of an AVT antiserum. (c) Effect of ASA (75 mg kg^{-1}) on arterial blood pressure (upper part, original recording in a single experiment) and on glomerular filtration rate (GFR, lower part, means with s.e. of 6 experiments) in water ducks during i.v. infusion of 1.6 ml min^{-1} of 250 mosm kg^{-1} NaCl solution. (d) Single experiment in a water duck made diuretic by i.v. infusion of 1.6 ml min^{-1} of 200 mosm kg^{-1} glucose solution: effect of i.v. injection of a mannitol solution of the same osmotic strength as the routinely i.v. injected ASA dose (75 mg kg^{-1}). (e) Single experiment in a water duck made diuretic by i.v. infusion of 1.6 ml min^{-1} of 200 mosm kg^{-1} glucose solution: effects of i.v. injections of AVT, first without and second after 90 min of continuous ASA infusion at a rate of 2.5 mg kg^{-1} min^{-1}.

Investigative studies

A number of experiments were carried out to obtain further preliminary information about the possible mode of ASA and SA action and about the proposed alteration of renal AVT sensitivity depending on the composition of the infused load. Figure 6 summarizes corresponding observations obtained in single experiments and small experimental series respectively.

Arginine vasotocin sensitivity of renal excretion

Our original presumption of an altered state of AVT sensitivity in ducks loaded with hyposmotic saline solution could be confirmed by experiments in which AVT was injected i.v. during steady state diuresis induced either by continuous i.v. infusion of 1.6 ml min^{-1} of 200 mosm kg^{-1} glucose solution or of 1.6 ml min^{-1} of 250 mosm kg^{-1} NaCl solution. As shown by Figure 6a, the maximum inhibition of urine flow rate in the glucose-infused ducks after single injections of 29 ng AVT approached the level of anuria. However, when similar urine flow rates had been established by saline infusion, the same AVT dose proved ineffective and only an approximately three fold dose of AVT produced a slight reduction of urine flow rate.

Despite the much reduced antidiuretic effect of AVT in ducks receiving a continuous load of hyposmotic saline, endogenous AVT, being present in the plasma at a considerable concentration (Table 2), nevertheless seems to exert some control on renal fluid excretion. Figure 6b shows a single experiment in which an AVT antiserum was given i.v. to a duck in steady state diuresis under continuous infusion of 1.6 ml min^{-1} of 250 mosm kg^{-1} NaCl solution. Binding of the circulating AVT clearly caused a temporarily enhanced diuresis. However, the rapid re-establishment of the previous urine flow rate cannot be ascribed to the restoration of the AVT plasma concentration to the level before antiserum injection, because other studies have shown that plasma AVT may remain at very low levels for several hours after anti-serum administration.

Acetylsalicylic acid effects

Figure 6c shows, by demonstrating the continuous recording of the arterial pulse pressure in a single animal, that ASA did not produce any significant changes at the dose usually employed in the present investigation. The lower part of Figure 6c further shows that GFR, as determined with the [^{14}C]-inulin clearance method (Dantzler, 1966) in 6 experiments was not affected by ASA.

Figure 6d experimentally confirms the conclusion, based on theoretical considerations, that ASA did not exert its influence on renal excretion through its non-specific property of being an osmotically active substance. Mannitol, a substance known to induce osmotic diuresis when administered to ducks in appropriate amounts (Simon-Opperman *et al.*, 1979), produced no effect whatsoever on the urine flow of a water duck receiving 1.6 ml min^{-1} of 200 mosm kg^{-1} glucose solution, when it was given in an amount osmotically equivalent to the standard dose of 75 mg kg^{-1} ASA.

Figure 6e shows a single experiment suggesting that ASA seems to interfere in some way with the action of AVT at the renal level. When 25 ng AVT were injected i.v. into a water duck made diuretic by i.v. infusion of 1.6 ml min^{-1} of 200 mosm kg^{-1} glucose solution, i.e. in a condition in which the duck reacts sensitively to AVT, a strong antidiuresis was induced, the animal becoming virtually anuric for 10 min. However, the same dose of AVT given some time later in the presence of ASA infused at a rate of 2.5 mg kg^{-1} min^{-1}, had a remarkably reduced effect.

Inhibition of prostaglandin synthetase?

In a number of studies on several mammalian species, the effects of ASA have been attributed to its property as an inhibitor of prostaglandin synthetase. Although the ASA effects found in the duck were basically opposite to those reported for mammals, it was felt necessary to investigate the question whether prostaglandin synthesis inhibition might be involved. Not shown by a graph or table, because of the basically negative results, are experiments with two other potent inhibitors of prostaglandin synthetase, indomethacin and sodium meclophenamate. Indomethacin at doses up to 50 mg kg^{-1} had no detectable effect upon renal water and salt excretion. However, because of solubility problems that required the use of a vehicle containing Tris and Tween 80, the indomethacin solution was badly tolerated by the birds and it often produced obvious discomfort including vomiting, although this was only of short duration. Sodium meclophenamate was tolerated by the animals without distress at doses up to 20 mg kg^{-1}; however, this drug also failed to produce consistent effects upon the renal functions monitored.

Discussion

The increase of urine flow rate in response to ASA in ducks under different conditions of salt and water loading (hyposmotic saline infusion in saltwater and freshwater adapted ducks, water loading in freshwater adapted ducks) contrasts with the general observation of an inhibitory effect on renal fluid excretion

in mammals (Berg & Bergan, 1976; Berg, 1977). A polyuric effect of ASA was reported to occur in dogs at 'toxic' levels ($> 400\ \mu g\ ml^{-1}$) of plasma concentration (Berg & Bergan, 1976). While the ASA plasma concentrations attained in the present study were at approximately that level with the usually employed dose of $75\ mg\ kg^{-1}$, it was equally clear that the relationship between the polyuric response and the amount of ASA infused was consistent from much lower to even higher doses. Nothing is known about the toxicity of salicylates in birds, and with the regular doses of ASA as well as SA applied in this study, no apparent toxic effects were observed. The highest routinely used ASA dose of $75\ mg\ kg^{-1}$ is described as being 'analgesic' in mammals (O'Dea *et al.*, 1975).

A clear difference may, thus, be stated between the effects of ASA on renal fluid excretion observed in the duck and in a number of mammals, for which there is general agreement that the ASA effects are due to its inhibitory action of prostaglandin formation. Evidence is derived from *in vitro* studies (Schlondorff *et al.*, 1981) as well as from comparative studies with other inhibitors of prostaglandin synthetases, indomethacin, meclophenamate, and others (Fejes-Tóth *et al.*, 1977). Inhibition of prostaglandin formation and/or action is thought to potentiate the effect of the antidiuretic hormone (ADH) resulting in oliguria and sodium retention, but the results are not completely consistent (Kirschenbaum & Stein, 1976). The mechanisms of action are not fully evaluated, multiple sites of interaction with AVP being inferred from *in vitro* studies (Schlondorff *et al.*, 1981). With regard to the ASA action on renal excretion in ducks, our own preliminary observations suggest that the potency of ASA to inhibit prostaglandin synthetases is not relevant. Indomethacin as well as meclophenamate had neither congruent nor antagonistic effects in comparison to ASA.

The only comparative information available for the action of salicylates on renal function in mammals and birds concerns salicylate (SA) itself. In contrast to ASA, SA was found to increase the renal output of water and salt in mammals at low (Ramsay & Elliot, 1967) and toxic (Quintanilla & Kessler, 1973) dose levels. This difference has been explained by species differences as well as by differences in dose and duration of observation. In addition, Berg & Bergan (1976) suggested that the different renal actions of ASA and SA may reflect their different potencies as inhibitors of prostaglandin synthetases. In the duck, the dose-response relationships for polyuria induced by sodium salicylate was closely similar to that for polyuria due to ASA. This might indicate that, in fact, ASA works via conversion to SA, its primary metabolite, a reaction which *in vivo* occurs very rapidly according to O'Dea *et al.* (1975) and to our own observations in ducks. The polyuria induced in ducks by ASA as well as SA appears, at the first glance, due to a diuretic action of the drugs. As shown by Figure 6e effects could be demonstrated that point to an AVT antagonism of ASA at the renal level. However, there are other observations which differ from those made in ducks on the occasion of clearly diuretic reactions. Usually, any short time diuretic response of a duck is associated with some, though only slight, increase of osmolal excretion, but this effect was quite pronounced during polyuria induced by ASA and SA respectively, so that the decreases in urine osmolality with increasing urine flow rate were small or even insignificant. As a consequence the correlation between urine flow rate and the logarithm of urine osmolality typical for the duck's renal excretion (Möhring *et al.*, 1980) was weakly expressed in the results of the present study (Table 3), either due to a very low slope or due to the lack of statistical significance. Thus, in addition to a seemingly antagonistic effect of the salicylates on AVT in ducks at the renal level, a saluretic effect of these drugs has to be taken into consideration, as indicated by the results summarized in Table 1. On the other

Table 3 Relationship between urine flow rate (Y) and the logarithm of urine osmolality (X) under the influence of $75\ mg\ kg^{-1}$ acetylsalicylic acid (ASA) as evaluated by linear regression analysis

I	Salt ducks with continuous i.v. infusion of $1.6\ ml\ min^{-1}$ NaCl solution of $250\ mosm\ kg^{-1}$	$Y = +0.014\,X + 2.25$ $n = 16,\ r = 0.135$
II	Water ducks with continuous i.v. infusion of $1.6\ ml\ min^{-1}$ NaCl solution of $250\ mosm\ kg^{-1}$	$Y = -0.044\,X + 2.48$ $n = 12,\ r = -0.790$
III	Water ducks with continuous i.v. infusion of $1.6\ ml\ min^{-1}$ glucose solution of $200\ mosm\ kg^{-1}$	$Y = +0.042\,X + 1.80$ $n = 12,\ r = 0.089$

hand, an osmotic component in the renal response of the ducks to salicylates appears unlikely, both theoretically and experimentally, as demonstrated by the observation shown in Figure 6d.

The reduced response of renal fluid excretion to exogenously administered AVT of ducks receiving ASA suggests some kind of interference of this drug with the renal action of the antidiuretic hormone. However, a direct, competitive antagonism seems to be unlikely according to a number of further observations. The greatly different sensitivities to AVT of renal excretion in ducks receiving hyposmotic saline or glucose infusions did not express itself in major differences of the responses to ASA. Further, changes of glomerular filtration rate, as they occur in birds under the influence of ADH (Ames *et al.*, 1971) were not observed with ASA. Maintenance of a high AVT level by exogenous hormone infusion also did not influence the ASA action.

With regard to a possible interference of ASA with the control of salt and water balance at the level of the CNS, the delayed response of the salt glands to ASA seem to exclude such an action. Further, the endogenous plasma level of AVT was not significantly altered by ASA injection.

Although some kind of interference of ASA and SA respectively, with AVT in its action on collecting duct water permeability is not excluded by the results of the present study, it may be assumed that such an action, if it exists, is not due to the potency of ASA in inhibiting prostaglandin synthetases. In addition to this questionable antagonistic action of ASA on AVT, however, it seems that ASA and SA respectively, caused an increase of the load of water and electrolytes to the distal segment of the nephron to a degree that could not be compensated for by the mechanisms of electrolyte and water reabsorption established at this level. Such an action would conform to the observation that urinary excretion of Na^+ and Cl^- as well as of K^+ increased in proportional fashion after ASA administration, resulting in an unchanged Na/K-quotient. This observation further seems to exclude that ASA acted by antagonizing the mineralocorticoid action, apart from the rapidity of the ASA action which would be difficult to reconcile with this mechanism. Thus, the possibility that ASA and SA respectively, acted at a more proximal site of the tubular system by reducing electrolyte reabsorption in general has to be considered. This is, in fact, a mode of action which has already been assumed to account for the effects of salicylates on the mammalian kidney (Ramsay & Elliot, 1967; Quintanilla & Kessler, 1973; Berg & Bergan, 1976).

The original question posed in the present study, the cause of the relative insensitivity of the saline loaded duck's kidney to AVT, has not been answered by the experimental results obtained. The assumed insensitivity of renal water excretion to AVT has, indeed, been confirmed in this study; however, contrary to what we had anticipated from studies in mammals, inhibition of prostaglandin synthesis appears not to potentiate AVT action in birds and thereby excludes the possibility that the observed insensitivity to AVT was mediated by prostaglandins. In addition, a polyuric action of ASA was observed in the duck which contrasted to the generally antidiuretic action of this drug in mammals and could not be ascribed to an interference with prostaglandin formation. In contrast to mammals, in which the inhibition of prostaglandin synthesis appears to constitute the prevailing effect of ASA on renal function, a direct effect of salicylates on tubular reabsorption appears as the prevailing mode of action of this drug in the kidneys of birds.

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