centrations of cAMP. Since SAM is currently being used to treat certain hepathopaties on the basis that this condition is accompanied with inhibition of the synthesis of SAM [11], the present results support the pharmacological use of SAM.

In summary, exogenous SAM can cross the cell membrane of isolated hepatocytes at pharmacological doses (concentrated ≥ 1 mM). At physiological concentrations SAM is either unable to cross the cell membrane or is rapidly metabolized. This argues against any physiological role for the uptake of SAM by hepatocytes. Our results are consistent with the data of Hoffman *et al.* [2], who concluded that the hepatocytes do not take up significant amounts of SAM when rat liver is perfused with 50 μM SAM.

Acknowledgements—We thank Cristina Paredes for typing the manuscript and Carlos Exposito for drawing the figures. I. V. is a fellow of Fondo de Investigaciones Sanitarias. This work was supported by grants from Europharma, Fondo de Investigaciones Sanitarias, and Comision Asesora para el Desarrollo de la Investigacion.

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REFERENCES

1. C. Pezzoli, G. Stramentinoli, M. Galli-Kienle and E. Pfaff, *Biochem. biophys. Res. Commun.* **85**, 1031 (1978).
2. D. R. Hoffman, D. W. Marion, W. E. Cornatzer and J. A. Duerre, *J. biol Chem.* **255**, 10822 (1980).
3. J. G. Castaño, S. Alemany, J. G. Nieto and J. M. Mato, *J. biol. Chem.* **255**, 9041 (1980).
4. J. G. Castaño, A. Nieto and J. E. Feliu, *J. biol. Chem.* **254**, 5576 (1979).
5. S. Alemany, I. Varela and J. M. Mato, *Biochem. J.* **208**, 453 (1982).
6. M. Garcia Gil, F. Alonso, V. Alvarez Chiva, M. Sanchez Crespo and J. M. Mato, *Biochem. J.* **206**, 67 (1982).
7. J. S. Schanche, T. Schanche and P. M. Ueland, *Biochim. biophys. Acta* **721**, 399 (1982).
8. I. Garcia-Castro, J. M. Mato, G. Vasanthakumar, W. P. Weismann, E. T. Schiffmann and P. K. Chiang, *J. biol. Chem.* **258**, 4345 (1983).
9. Y. Morita, R. P. Siraganian, C. K. Tang and P. K. Chiang, *Biochem. Pharmac.* **31**, 2111 (1982).
10. J. M. Mato, D. Pencev, G. Vasanthakumar, E. T. Schiffmann and I. Pastan, *Proc. natn. Acad. Sci. U.S.A.* **80**, 1929 (1983).
11. J. H. Horowitz, E. B. Rypins, J. M. Henderson, S. B. Heymsfield, S. D. Moffit, R. P. Bain, R. K. Chawle, J. C. Bleier and D. Rudman, *Gastroenterology* **81**, 668 (1981).

Biochemical Pharmacology, Vol. 33, No. 9, pp. 1564–1566, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

The effect of urine pH on the reduction of urinary PGE_2 excretion by indomethacin

(*Received* 22 *September* 1983; *accepted* 17 *November* 1983)

Urinary prostaglandin excretion has been regarded as an index of renal prostaglandin synthesis [1], and the urinary excretion of prostaglandin E_2 (PGE_2) has recently been used as a means of comparing the inhibition of prostaglandin synthesis elicited by different non-steroidal anti-inflammatory drugs [2]. There are several reasons why such comparisons between different agents can be misleading: it is widely recognized that changes in urine flow can influence prostaglandin excretion [3] but there are also other important determinants of prostaglandin excretion, notably the urinary pH [4, 5]. Thus if different non-steroidal anti-inflammatory agents change urine pH to different extents, this could alter urinary prostaglandin excretion in a manner not necessarily related to the extent of prostaglandin synthesis inhibition. In the present paper, we report the effects of a single non-steroidal anti-inflammatory agent, indomethacin, on the urinary excretion of PGE_2 at different urinary pH values.

Materials and methods

PGE_2 was obtained from the Upjohn Co. (Kalamazoo, MI) and [^{3}H]PGE_2 from Amersham International. (Amersham, Bucks., U.K.). PGE_2 antiserum was from Miles-Yeda (Rehovot, Israel). Silicic acid for column chromatography was from the Sigma Chemical Co. (St. Louis, MO).

Experimental protocol. All experiments were performed on conscious female Wistar rats (weight range 200–350 g), which were allowed free access to food and water prior to the experiments. Rats were placed in individual metabolic cages at the same time on each of 2 days, a control day and an experimental (indomethacin) day, and urine was collected over a 3 hr period. Three groups of animals were used; the animals in each group received, by stomach tube on each of the 2 days, the following solutions at the start of the urine collection period: group A, 0.9% NaCl (2 ml/100 g body wt); group B, 1% $NaHCO_3$ (2 ml/100 g body wt); group C, 1.3% NH_4Cl, 0.3% NaCl (3 ml/100 g body wt). On the experimental day each animal received indomethacin (10 mg/kg body wt, i.p.) at the start of the urine collection period.

Assays. The pH of each urine sample was measured, and the urine flow was determined. The samples were stored at −20° while awaiting radioimmunoassay for PGE_2. The assay procedure has been described elsewhere [5, 6].

Statistical methods. The significance of differences in the measured variables between the control and experimental days was assessed using Student's *t*-test. Results are presented as means ± S.E.

Results

The three groups of rats (A, B, C) were intended to have normal, alkaline and acidic urines, respectively (in the rat, urine pH is typically approximately 6.0). The urine pH values achieved are shown in Fig. 1. It is clear that on the control days, urine pH correlates well with PGE_2 output (Fig. 1), PGE_2 output being high at high pH (group B) and low at low pH (group C), with group A being intermediate for both pH and PGE_2 output.

Indomethacin administration had no significant effect on the pH of any of the groups. However, indomethacin did elicit a marked reduction in PGE_2 output in the group with alkaline urine (B) and the group with normal urine (A), but in group C (acid urine), where PGE_2 excretion was

Table 1. Urine flow, sodium output and urine osmolality in the three groups of rats before (control) and after (indo) indomethacin administration

		Urine flow (μl/min)	Sodium output (μmole/min)	Urine osmolality (mosm/kg H_2O)
Group A	Control (12)	25.6 ± 1.9	2.21 ± 0.20	515 ± 23
	Indo (12)	14.9 ± 2.2	1.69 ± 0.18	1041 ± 96
Group B	Control (12)	26.9 ± 4.1	2.33 ± 0.17	509 ± 23
	Indo (12)	17.6 ± 1.0	1.77 ± 0.18	652 ± 57
Group C	Control (6)	26.8 ± 3.1	2.30 ± 0.29	604 ± 33
	Indo (10)	23.0 ± 3.2	1.90 ± 0.35	680 ± 61

already low, indomethacin administration did not lead to a further reduction.

The urine flow was almost identical in the three groups on the control day (Table 1), so that clearly the differences in PGE_2 excretion cannot be attributed to flow differences. Indomethacin elicited significant reductions in urine flow in groups A ($P < 0.01$) and B ($P < 0.01$), but in group C, although mean flow fell slightly, the change was not significant (Table 1).

Discussion

The present findings confirm our previous observations which indicate that urinary pH has a marked effect on urinary PGE_2 excretion [4, 5]. The most likely explanation for this finding is that since prostaglandins are weak acids, the tubular fluid pH will determine the extent of prostaglandin ionization, and only the unionized forms are readily diffusible [7]. Thus at high urine pH values, reflecting high collecting tubule pH, PGE_2 will be unable to diffuse out of the nephron and hence will be excreted in increased amounts.

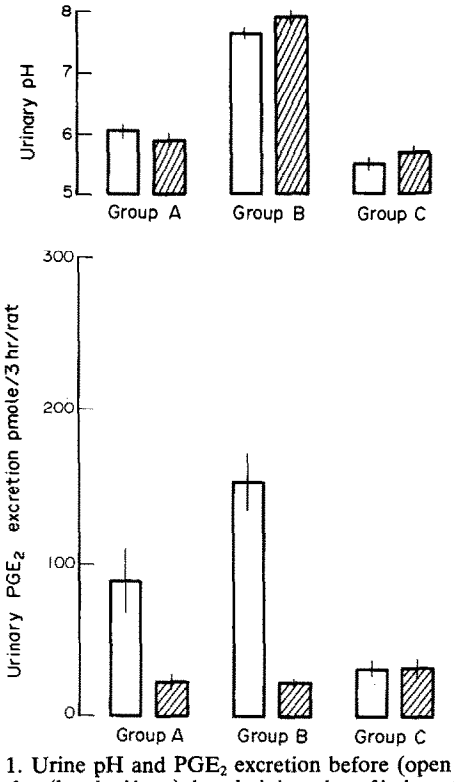

Fig. 1. Urine pH and PGE_2 excretion before (open bars) and after (hatched bars) the administration of indomethacin (10 mg/kg body wt, i.p.). Indomethacin reduced PGE_2 output to values which were not significantly different between the groups, although the initial (control) values differed greatly. Group A received (orally) 0.9% NaCl (2 ml/100 g body wt); group B received 1% $NaHCO_3$ (2 ml/100 g body wt), and group C received 1.3% NH_4Cl, 0.3% NaCl (3 ml/100 g body wt). The administration of these solutions was designed to elicit similar changes in urine flow and sodium output, whilst producing widely different urine pH values.

However, the main point we wish to make from the present experiments is that measurements of 'percentage inhibition' of prostaglandin synthesis by non-steroidal anti-inflammatory drugs are of little value, since the percentage inhibition observed depends on the initial prostaglandin output rather than on the post-inhibitor value. Thus in our experiments it appears that there is a residual output of PGE_2, approximately 25 pmole/3 hr per rat, which cannot be abolished by 10 mg/kg body wt indomethacin—but this level will be attained whatever the initial PGE_2 excretion. Thus the 'percentage inhibition' of PGE_2 synthesis in the three groups (A, B, C) is respectively, 76, 86, and 0%.

It is remotely conceivable that part (or even all) of the residual PGE_2 excretion which cannot be inhibited by indomethacin could be due to some other urinary constituent which reacts in the radioimmunoassay. However, this seems very unlikely as the urine samples were extracted into organic solvent, the E-prostaglandins were separated by column chromatography prior to the assay, and the antiserum has high specificity.

It seems clear that comparisons of the effects of non-steroidal anti-inflammatory drugs (NSAIDS) on urinary PGE_2 output are not meaningful unless it is known that the urine pH is identical in different groups before the administration of such agents; such comparisons may also be complicated by the effects of the NSAIDS themselves on urine pH.

Finally, it should be pointed out that different anti-inflammatory agents have different latencies in the onset of their effects on urinary PGE_2 output, even when administered intravenously (C. J. Lote, unpublished observation), and this factor is an additional one which makes comparisons between agents difficult.

Summary

Urinary PGE_2 excretion is influenced by urinary pH, being higher at high pH values. Indomethacin (10 mg/kg body wt, i.p.) was found to reduce urinary PGE_2 excretion to the same absolute value irrespective of the initial PGE_2 output. It is concluded that measurements of 'percentage inhibition' of urinary PGE_2 excretion by non-steroidal anti-inflammatory agents must be interpreted with caution.

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REFERENCES

1. J. C. Frölich, T. W. Wilson, B. J. Sweetman, M. Smigel, A. S. Nies, K. Carr, J. T. Watson and J. A. Oates, *J. clin. Invest.* **55**, 763 (1975).
2. K. Matsuda, K. Ohnishi, E. Misaka and M. Yamazaki, *Biochem. Pharmac.* **32**, 1347 (1983).
3. M. Kirschenbaum and J. Stein, *J. Lab. clin. Med.* **90**, 46 (1977).
4. J. Haylor and C. J. Lote, *J. Physiol.* **338**, 62P (1983).
5. J. Haylor and C. J. Lote, *Clin. Sci.* (in press).
6. C. J. Lote, A. McVicar and A. Thewles, *J. Physiol.* **336**, 39 (1983).
7. L. Z. Bito, M. Wallenstein and E. Baroody, *Adv. Prostaglandin Thromboxane Res.* **1**, 297 (1976).

Biochemical Pharmacology, Vol. 33, No. 9, pp. 1566–1568, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

Studies of the α_2-adrenoceptor affinity and the α_2- to α_1-adrenoceptor selectivity of some substituted benzoquinolizines using receptor-binding techniques

(*Received* 10 *September* 1983; *accepted* 17 *November* 1983)

There is now considerable evidence that α-adrenoceptors exist as two pharmacologically distinct subtypes designated α_1 and α_2. Support for this classification has come from functional studies as well as from direct receptor labelling techniques [1]. Therapeutic applications of selective α_2-adrenoceptor blockers remain, as yet, a matter for speculation.

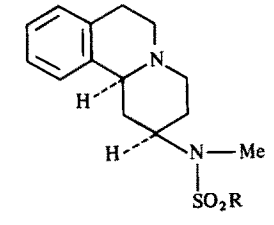
Recently, a group of substituted benzoquinolizine adrenoceptor blocking agents (Wy 25309, Wy 26392 and Wy 26703) has been identified as having greater affinity for α_2- than α_1-adrenoceptors using isolated tissue preparations [2]. The compounds have been reported to have less 5-hydroxytryptamine antagonist potency than yohimbine [3]. The purpose of the present study was to investigate further the α-adrenoceptor selectivity of these compounds using radioligand binding methods. The α_2-adrenoceptor blockers yohimbine [4] and RX 781094 [5] were included for comparison. The structures of these compounds are shown in Fig. 1.

α_1-Adrenoceptor affinity was determined from the ability of the antagonists to displace [^{3}H]prazosin, an α_1-selective ligand [6], from binding sites in rat cerebral cortex membrane fractions. [^{3}H]Rauwolscine, an α_2-selective ligand [7, 8], was similarly used to assess α_2-adrenoceptor affinity in membrane fractions of rat cerebral cortex, rat kidney cortex and of lysed human blood platelets.

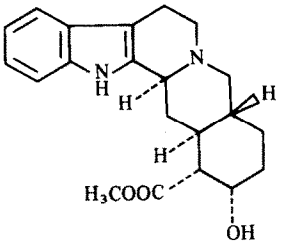
Materials and methods

Preparation of rat cerebral cortex and kidney cortex membranes. Cerebral cortex or kidney cortex tissue obtained from male Wistar rats (150–250 g) was homogenized in 20 vols. of ice-cold 5 mM Tris–HCl, 5 mM EDTA buffer with an Ultra-Turrax Homogeniser for 2 × 10 sec bursts. The suspension was then centrifuged at 27,000 *g* for 10 min at 4°. The resulting pellet was washed once more with the same buffer by resuspension, followed by centrifugation at 27,000 *g* for 10 min at 4°. One final washing was carried out by resuspending the pellet in ice-cold 'assay buffer' (50 mM Tris–HCl, 0.5 mM EDTA, 0.1% ascorbate, pH 7.5) and then centrifuging at 27,000 *g* for 10 min at 4°. The final pellet was resuspended in the appropriate volume of assay buffer (see below).

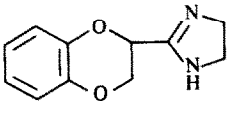
Preparation of human platelet membrane lysates. Blood was collected from human volunteers with 3% (w/v) sodium citrate as anticoagulant. Platelet-rich plasma (PRP) was obtained by centrifuging the collected blood at 270 *g* for 15 min at 10°. PRP was then centrifuged at 27,000 *g* for 10 min at 4° and the resulting pellet resuspended in ice-cold 'lysing buffer' (5 mM Tris–HCl, 5 mM EDTA) and left for 1–2 min before being homogenized for 10 strokes with a motor-driven glass–Teflon homogeniser. The suspension was then centrifuged at 27,000 *g* for 10 min at 4°. The pellet was washed once with lysing buffer by gentle resuspension



R
Wy 25309 – CH_3
Wy 26392 – $CH_2CH_2CH_3$
Wy 26703 – $CH_2CH(CH_3)_2$



Yohimbine



RX 781094

Fig. 1. Structures of the α-adrenoceptor blockers.