Effect of cyclo-oxygenase blockade on the renal actions of vasopressin and SK&F 105494 in the rhesus monkey

¹David P. Brooks, Nancy C. Caldwell, Paul F. Koster, Christine R. Albrightson-Winslow & Lewis B. Kinter

SmithKline Beecham Pharmaceuticals, Department of Pharmacology, L521, P.O. Box 1539, King of Prussia, PA 19406-0939, U.S.A.

1 Vasopressin administration to pentobarbitone-anaesthetized, hydrated female rhesus monkeys resulted in dose-dependent increases in urine osmolality and decreases in urine flow. Treatment with indomethacin at a dose (5 mg kg^{-1} , i.v.) that reduced urinary prostaglandin E_2 (PGE_2) excretion by at least 70% did not alter these responses.

2 The vasopressin antagonist, SK&F 105494 ($\text{PAs}^{1,6}\text{D-Tyr}(\text{Et})^2\text{Val}^4\text{Arg}^7\text{D-Arg}^8\text{desGly}^9$ arginine vasopressin; $10 \mu\text{g kg}^{-1}$), caused significant rightward shifts ($P < 0.05$) of both the vasopressin-urine osmolality and the vasopressin-urine flow dose-response curves. Treatment with indomethacin did not alter these responses.

3 SK&F 105494 alone or after indomethacin treatment had minimal effects on urine osmolality and urine flow.

4 The data indicate that indomethacin does not alter the antidiuretic activity of vasopressin in the rhesus monkey and that SK&F 105494 is a potent antagonist of exogenous vasopressin with minimal agonist activity.

Introduction

Inhibition of cyclo-oxygenase activity has been shown to augment the antidiuretic activity of vasopressin in the rat (Berl *et al.*, 1977) and the dog (Anderson *et al.*, 1975; Fejes-Toth *et al.*, 1977; Albrightson-Winslow *et al.*, 1989). Indomethacin and meclofenamate unmask the antidiuretic partial agonist activity of the vasopressin antagonist, SK&F 101926, in the dog (Albrightson-Winslow *et al.*, 1989). On the other hand, indomethacin appears not to enhance vasopressin antidiuretic activity in man (Padfield & Grekin, 1981) and SK&F 101926 demonstrates full agonist activity as an antidiuretic in man without prior cyclo-oxygenase inhibition (Allison *et al.*, 1988). SK&F 101926 is also a full antidiuretic agonist in the absence of cyclo-oxygenase blockade in the rhesus monkey (Brooks *et al.*, 1988a). Another vasopressin antagonist, SK&F 105494, had minimal antidiuretic agonist activity in the rhesus monkey and produced a water diuresis in hydropenic animals (Brooks *et al.*, 1988a). One of the implications of these findings is that in the rhesus monkey, as in man, prostaglandins may not modulate vasopressin antidiuretic activity. In the present study, therefore, we have tested the ability of SK&F 105494 to inhibit exogenous vasopressin activity in the rhesus monkey and evaluated the effect of cyclo-oxygenase inhibition on the renal actions of both vasopressin and SK&F 105494.

Methods

Vascular-Access-Ports (Norfolk Medical Products) were inserted into a femoral vein of 3 female rhesus monkeys (*Macaca mulatta*; 5–8 kg). Ports were flushed twice a week and filled with a heparin (500 u ml^{-1}) saline (0.9%) solution. Before each experiment, animals were deprived of food but not water for 16 h. On the morning of the experiment, catheters were placed in the bladder (Foley Pediatric 08 FR) and the animals were lightly anaesthetized with sodium pentobarbitone. Animals were placed on circulating water heating pads (Aquamatic model K-20), an infusion of hypotonic dextrose (3%) was initiated at $0.5 \text{ ml kg}^{-1} \text{ min}^{-1}$ and urine collected at

10 min intervals. When urine osmolality had decreased to $50 \text{ mOsm kg}^{-1} \text{ H}_2\text{O}$ either indomethacin (5 mg kg^{-1} , i.v.) or vehicle was administered followed 30 min later by either SK&F 105494 ($\text{PAs}^{1,6}\text{D-Tyr}(\text{Et})^2\text{Val}^4\text{Arg}^7\text{D-Arg}^8\text{desGly}^9$ arginine vasopressin; $10 \mu\text{g kg}^{-1}$, i.v.) or vehicle. Ten min urine collections were taken for the next 90 min. A second dose of vehicle or SK&F 105494 was administered and then an arginine vasopressin dose-response curve was generated. Arginine vasopressin (0.3 to 100 ng kg^{-1}) was administered in a cumulative manner and urine collections made at 5 min intervals. The infusion rate of dextrose was adjusted to equal urine output throughout the experiment. Blood pressure was monitored indirectly at 5 min intervals using a Dynamap (Criterikon). Each animal underwent all of the 4 protocols with at least a 2 week interval between each procedure.

Urinary prostaglandin E_2 (PGE_2) excretion was determined, before and after indomethacin treatment, by radioimmunoassay after extraction of PGE_2 from acidified (pH 3.4–4) urine by methods described previously (Albrightson *et al.*, 1989). Data are expressed as mean $\pm$ s.e.mean. Dose-response curves were compared by calculating either the ED_{200} (dose of vasopressin that increased urine osmolality to $200 \text{ mOsm kg}^{-1} \text{ H}_2\text{O}$) or $\text{ED}_{1.0}$ (dose of vasopressin that decreased urine flow to 1.0 ml min^{-1}). The ED_{200} and $\text{ED}_{1.0}$ values were transformed logarithmically because of non-homogeneity of variance and compared statistically by a paired *t* test. Values are given as mean $\pm$ s.e.mean.

Results

Arginine vasopressin administration resulted in dose-dependent increases in urine osmolality and decreases in urine volume (Figure 1). Indomethacin pretreatment did not significantly alter urine osmolality or volume responses (Figure 1); $\text{ED}_{200} = 6.3 \pm 2.4$ and $6.3 \pm 3.1 \text{ ng kg}^{-1}$ and $\text{ED}_{1.0} = 1.5 \pm 0.2$ and $1.3 \pm 0.6 \text{ ng kg}^{-1}$ in vehicle- and indomethacin-treated monkeys respectively ($n = 3$ per group). SK&F 105494 produced a significant ($P < 0.05$) rightward shift in the urine osmolality and urine flow responses to vasopressin and these shifts were not altered by indomethacin (Figure 1); $\text{ED}_{200} = 61.7 \pm 25.9$ and $36.3 \pm 12.3 \text{ ng kg}^{-1}$ and $\text{ED}_{1.0} = 8.7 \pm 3.2$

¹ Author for correspondence.

Figure 1 The effects of vehicle ($\square$), indomethacin ($\circ$, 5 mg kg^{-1}), SK&F 105494 ($\blacksquare$, $10 \mu\text{g kg}^{-1}$) or a combination of indomethacin and SK&F 105494 ($\bullet$) on changes in urine osmolality (a) and urine flow (b) induced by increasing doses of vasopressin (AVP). * $P < 0.05$, SK&F 105494 vs. vehicle.

Figure 2 (a) Urinary prostaglandin E_2 (PGE_2) concentration (UPGE_2) and (b) urinary PGE_2 excretion (UPGE_2V) before (Time 0) and after administration of indomethacin (5 mg kg^{-1}). ** $P < 0.01$ vs. Time 0.

and $9.0 \pm 2.7 \text{ ng kg}^{-1}$ in vehicle- and indomethacin-treated monkeys respectively ($n = 3$ per group).

SK&F 105494 administration, in either the presence or absence of indomethacin, did not alter significantly either urine osmolality (44 ± 4 and $42 \pm 5 \text{ mOsm kg}^{-1} \text{H}_2\text{O}$ at 60 min in vehicle- and indomethacin-treated groups, respectively) or urine flow (2.2 ± 0.2 and $2.2 \pm 0.3 \text{ ml min}^{-1}$ at 60 min, respectively). In 1 animal, however, there was a transient reduction in urine flow within 20 min of administration of SK&F 105494 which was associated with a transient reduction in blood pressure (62 to 42 mmHg). However, both parameters returned to control levels at 60 min.

Urinary excretion of prostaglandin E_2 (PGE_2) was $54 \pm 19 \text{ pg min}^{-1}$ before indomethacin treatment (5 samples from 3 monkeys). At 30 and 120 min following indomethacin administration, the PGE_2 concentration in 8 of 10 urine samples fell below the detection limit of the assay ($2.5 \text{ pg } 100 \mu\text{l}^{-1}$), indicating that the urinary PGE_2 excretion had been reduced by at least 60 and 70%, respectively (Figure 2).

Discussion

In the present study, we demonstrated for the first time that in the rhesus monkey SK&F 105494 is a potent inhibitor of exogenous vasopressin activity. Furthermore, indomethacin, in a dose that reduced urinary prostaglandin excretion by at least 70%, failed to alter vasopressin antidiuretic activity either in the presence or absence of receptor blockade. This is in contrast to observations made in rats and dogs where cyclo-oxygenase inhibition enhances vasopressin antidiuretic activity (Anderson *et al.*, 1975; Berl *et al.*, 1977; Fejes-Toth *et al.*, 1977; Albrightson-Winslow *et al.*, 1989) but is similar to what is observed in man (Padfield & Grekin, 1981). Our assay system did not permit us to observe a greater than 70% reduction in urinary prostaglandin excretion, suggesting that some residual production may be present. However, we do not believe that this is likely, since this dose of indomethacin reduced the PGE_2 concentration in 8 of 10 urine samples to below the detection limit of the assay and, in dogs, a smaller dose of indomethacin reduced PGE_2 levels in the urine by 80–90% and shifted the vasopressin dose-response curve (Albrightson-Winslow *et al.*, 1989). The results suggest that in the rhesus monkey, as in man, renal prostaglandin production may not modulate the antidiuretic activity of vasopressin. These observations are consistent with the observation that indomethacin can unmask the agonist activity of the vasopressin antagonist SK&F 101926 in the dog (Albrightson-Winslow *et al.*, 1989); whereas, in both man (Allison *et al.*, 1988) and rhesus monkey (Brooks *et al.*, 1988a), this agent demonstrated full agonist activity without prior cyclo-oxygenase inhibition. SK&F 105494 demonstrated minimal agonist activity in the rhesus monkey, even in the presence of indomethacin. Furthermore, the ability of SK&F 105494 in the rhesus monkey to inhibit the antidiuretic activity of exogenous vasopressin was also unaffected by indomethacin treatment. We have previously shown that SK&F 105494 can cause a water diuresis in the hydropenic rhesus monkey (Brooks *et al.*, 1988a).

This study further indicates the possible utility of the rhesus monkey as a model for renal vasopressin agonist and antagonist activity in man. Previously, we have also shown that the stereochemical requirements of vasopressin antagonism are similar for *in vitro* activity in human renal tissue and both *in vitro* and *in vivo* activity in rhesus monkey (Brooks *et al.*, 1989). The rhesus monkey also appears to be a good model for the flushing activity associated with vasopressin V_2 agonists (Brooks *et al.*, 1988b).

We are grateful to S. Campbell, E. Jenkins, C. Kenney and J. Kissinger for excellent animal handling and care; Dr A. Nichols for helpful discussion; and S. Tirri for assistance in preparing the manuscript.

References

- ALBRIGHTSON-WINSLOW, C.R., CALDWELL, N., BROOKS, D.P., HUFFMAN, W., STASSEN, F.L. & KINTER, L.B. (1989). Cyclooxygenase inhibition unmasks the full antidiuretic activity of the vasopressin antagonist, SK&F 101926, in dogs. *J. Pharmacol. Exp. Ther.*, **249**, 366–371.
- ALLISON, N., ALBRIGHTSON-WINSLOW, C., BROOKS, D.P., STASSEN, F.L., HUFFMAN, W.F., STOTE, R.M. & KINTER, L.B. (1988). Species heterogeneity and antidiuretic hormone antagonists: what are the predictors? In *Vasopressin, Cellular and Integrative Functions*, ed. Cowley, A.W., Liard, J.F. & Ausiello, D. pp. 207–214. New York: Raven Press.
- ANDERSON, R.J., BERL, T., McDONALD, K.M. & SCHRIER, R.W. (1975). Evidence for an in vivo antagonism between vasopressin and prostaglandin in the mammalian kidney. *J. Clin. Invest.*, **56**, 420.
- BERL, T., RAZ, A., WALD, H., HOROWITZ, J. & CZACZKES, W. (1977). Prostaglandin synthesis inhibition and the action of vasopressin: studies in man and rat. *Am. J. Physiol.*, **232**, F529.
- BROOKS, D.P., KOSTER, P.F., ALBRIGHTSON-WINSLOW, C.R., STASSEN, F.L., HUFFMAN, W. & KINTER, L.B. (1988a). SK&F 105494 is a potent vasopressin antidiuretic antagonist in the rhesus monkey (*Macaca mulatta*). *J. Pharmacol. Exp. Ther.*, **245**, 211–215.
- BROOKS, D.P., KOSTER, P., ALBRIGHTSON, C.R., STASSEN, F.L., HUFFMAN, W.F., MOORE, M., SCHMIDT, D. & KINTER, L.B. (1989). Vasopressin receptor antagonism in rhesus monkey and man: stereochemical requirements. *Eur. J. Pharmacol.*, **160**, 159–162.
- BROOKS, D.P., KOSTER, P., STASSEN, F.L., ALBRIGHTSON-WINSLOW, C.R., HUFFMAN, W.F., WASSERMAN, M.A. & KINTER, L.B. (1988b). Flushing and haemodynamic responses to vasopressin peptides in the rhesus monkey. *Br. J. Pharmacol.*, **94**, 759–764.
- FEJES-TOTH, G., MAGYAR, A. & WALTER, J. (1977). Renal response to vasopressin after inhibition of prostaglandin synthesis. *Am. J. Physiol.*, **232**, F416.
- PADFIELD, P.L. & GREKIN, R.J. (1981). The effect of indomethacin on vasopressin-induced antidiuresis in man. *Clin. Sci.*, **61**, 493.

(Received November 13, 1989)

Accepted November 30, 1989)