The effect of glucocorticosteroids on *in vitro* motility of the ureter of the sheep

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- 1 The effects of three glucocorticosteroids, hydrocortisone, methylprednisolone and dexamethasone, as well as two non-steroidal anti-inflammatory agents (NSAIDs) indomethacin and diclofenac sodium were tested *in vitro* on the spontaneously rhythmic contracting ureteral preparation of the sheep.
- 2 The NSAIDs and the steroids methylprednisolone (10^{-7} – 10^{-4} M) and dexamethasone (10^{-8} – 10^{-4} M), dose-dependently inhibited ureteral motility. Hydrocortisone caused a cessation of spontaneous contractions only at the high concentration of 10^{-4} M.
- 3 Pretreatment of ureteral strips with the protein synthesis inhibitor cycloheximide (10^{-6} M) abolished the inhibitory action of the corticosteroids on peristalsis, consistent with the suggestion that the action of steroids on the ureter is mediated via the synthesis of the anti-phospholipase protein, lipocortin.
- 4 The potency of the steroids in descending order was found to be dexamethasone > methylprednisolone > hydrocortisone.

Introduction

A number of non-steroidal anti-inflammatory drugs (NSAIDs) have recently been shown to be efficacious in the clinical management of acute renal colic (Holmlund & Sjödin, 1978; Lundstam *et al.*, 1982). The pain relief produced by these agents may be due to inhibition of prostaglandin synthesis, resulting in intrarenal vasoconstriction, decreased urine production and consequently reduced pelvic pressure (Sjödin & Holmlund, 1982) as well as an analgesic action. The NSAIDs indomethacin and diclofenac sodium (Thulesius & Angelo-Khattar, 1985; Angelo-Khattar *et al.*, 1985) dose-dependently inhibit the intrinsic rhythmic activity of ureteral smooth muscle *in vitro*, ultimately resulting in a complete cessation of motility. This is associated with a reduction in the synthesis of prostaglandin $F_{2\alpha}$, prostacyclin and thromboxane A_2 ; therefore, the clinical effects can be associated with a spasmolytic action on the ureter (Thulesius *et al.*, 1987).

To date the potential use of glucocorticosteroids in the treatment of ureteral colic has been overlooked. Glucocorticosteroids inhibit the phospholipase A_2 enzyme which catalyses the release of arachidonic acid from membrane phospholipids and as such would also be expected to have a spasmolytic action on the ureter by virtue of a prostaglandin-inhibitory action. The aim of the

present study was to ascertain whether steroids block ureteral motility and to compare their efficacy to NSAIDs. The mechanism of steroid action on the isolated ureteral preparation was also investigated. Some results of this study were presented in preliminary form to a meeting of the British Pharmacological Society (Angelo-Khattar & Thulesius, 1987).

Methods

The specimens were obtained from Australian Merino sheep. Kidneys with attached ureters were harvested early in the morning from the local abattoir. The specimens were put into chilled Krebs-Henseleit solution and transported in thermos flasks to the laboratory. The ureters were dissected free of connective tissue and fat and 4 mm long rings were cut from the proximal part. These preparations were suspended vertically in 10 ml thermostated organ baths filled with Krebs-Henseleit solution at 37°C, bubbled with 95% O_2 and 5% CO_2 . The rings were attached to the bottom of the organ bath and the upper end connected with a Bioscience UFI force transducer. Isometric tension was continuously recorded on a Gilson 5/6 five-channel recorder. After suspension of the rings a starting pre-tension of 2 g

Figure 1 *In vitro* recording of rhythmic contractions of sheep ureteral rings (recording interrupted for 35 min). Upper panel: dexamethasone (10^{-5} M) (Dexa) added at arrowhead. Note intermittent contractions 40 min after drug administration. Middle panel: dexamethasone 10^{-5} M together with cycloheximide 10^{-6} M (Cyclo). Note: no inhibition of rhythmic contractions. Lower panel: control recording without drugs.

was obtained by adjustment with a micrometer screw. When the preparations were allowed to equilibrate, phasic rhythmic contractions started spontaneously. Sixty minutes after mounting when a stable pattern of contractions was established, one drug at a single concentration was added to the bath and the time until peristalsis ceased was measured. This parameter was designated as 'stop time' and used for

Figure 2 Relationship between concentration of corticosteroids (Hy = hydrocortisone, Me = methylprednisolone, Dx = dexamethasone), non-steroidal anti-inflammatory drugs (In = indomethacin, Di = diclofenac sodium) and time after administration of drug until cessation of phasic contractions = stop time. Graphs are regression lines calculated for 4 doses of each drug: $y = -3.3973 - 0.224x^{***}$ (for indomethacin); $y = -3.8619 - 0.224x^{**}$ (for diclofenac sodium); $y = -3.3190 - 0.032x^{**}$ (for dexamethasone); $y = -2.2755 - 0.023x^{**}$ (for methylprednisolone); $y = -2.0619 - 0.011x$ (for hydrocortisone). $^{**}P < 0.01$; $^{***}P < 0.001$.

subsequent assessment of drug effects. Responses were obtained to the NSAIDs indomethacin 10^{-8} – 10^{-4} M and diclofenac sodium 10^{-8} – 10^{-4} M and to the steroids dexamethasone sodium phosphate 10^{-7} – 10^{-4} M, methylprednisolone sodium succinate 10^{-7} – 10^{-3} M and hydrocortisone sodium succinate 10^{-5} – 10^{-3} M. In addition the effect of dexamethasone (10^{-5} M) after cycloheximide pretreatment (10^{-6} M) was investigated.

Drugs and chemicals

The bathing medium, Krebs-Henseleit solution contained (mM): NaCl 115.3, KCl 4.6, CaCl_2 2.3, MgSO_4 1.2, NaHCO_3 22.1, KH_2PO_4 1.1 and glucose 7.8 and was maintained at pH 7.4.

The following were used: indomethacin (Confortid, 50 mg vials, Dumex A/G, Copenhagen, Denmark); diclofenac Na (Voltaren, Ciba-Geigy Ltd., Basle, Switzerland); dexamethasone as dexamethasone sodium phosphate ampoules (Oradexon, N.V. Organon, OSS, Holland); methylprednisolone (methylprednisolone sodium succinate vials, Solu-medrol, Upjohn, Puurs, Belgium); hydrocortisone sodium succinate vials (Solu-cortef, Upjohn, Puurs, Belgium); cycloheximide (BDH Chemicals Ltd., Poole, England).

Calculations

For each concentration of NSAID and steroid, 6–15 experiments were performed and means calculated. For determination of potency of the individual drugs EC_{50} values were calculated from the dose-effect relationship of stop-times versus log molar concentration calculated by regression analysis based on simple linear regression, $y = a + bx$ where y = stop time in min, x = log dose in mM, a = constant, b = regression coefficient. The method has previously been applied by Bhargava & Thulesius (1987). Mean value $\pm$ s.e. of all the data are shown. Standard statistical methods were used for the calculations of means, standard deviation and standard error.

Results

The normal contractile pattern of the sheep ureteral preparation and the effect of the various prostaglandin synthesis inhibitors is shown in Figure 1. After a latency of 10–30 min, phasic rhythmic contractions started spontaneously and could be maintained for 5 h. A decline and ultimately complete cessation of activity was seen earlier in the presence of corticosteroids than in controls. The effect of the two NSAIDs, indomethacin and diclofenac Na, and the

Figure 3 Bar graphs showing mean values for time required for inhibition of spontaneous motility of the sheep ureter (stop times, min) at drug concentration of 10^{-5} M. Control experiments, $n = 10$; indomethacin, $n = 15$; diclofenac Na, $n = 10$; dexamethasone, $n = 8$; methylprednisolone, $n = 8$; hydrocortisone, $n = 6$; cycloheximide, $n = 10$. *** $P < 0.001$; ** $P < 0.01$.

three corticosteroids, hydrocortisone, methyl-prednisolone and dexamethasone, was measured by determining the interval (in min) from drug administration until cessation of rhythmic activity (stop time). Dexamethasone (10^{-8} – 10^{-4} M) and methyl-prednisolone (10^{-7} – 10^{-4} M) dose-dependently blocked ureteral motility. Hydrocortisone had an inhibitory action only at the high concentration of 10^{-4} M. Addition of cycloheximide (10^{-6} M) reversed the inhibitory action of the most potent steroid, dexamethasone. Figure 2 shows the relationship between the log molar concentration of the three corticosteroids and two NSAIDs versus stop-time in minutes. The graph was obtained by calculating regression lines for stop-time against molar concentration of the drugs. Figure 3 shows stop times for the control situation, the three corticosteroids, cycloheximide and two NSAIDs at a fixed drug concen-

tration of 10^{-5} M. From this the differential action of the various drugs can be seen. The interesting point in this graph is the fact that cycloheximide itself prolonged the duration of spontaneous ureteral mobility.

Discussion

Spontaneous peristaltic contractions are responsible for the transport of urine by the ureter and there is increasing evidence to suggest that this rhythmicity is initiated and maintained by the local release of prostaglandins (Al-Ugaily *et al.*, 1986; Thulesius *et al.*, 1986). Significant amounts of these prostanoids namely $\text{PGF}_{2\alpha}$, PGI_2 , thromboxane A_2 (TXA_2) were found to be released during ureteral peristalsis and treatment with cyclo-oxygenase blockers was shown to inhibit both ureteral motility (Thulesius & Angelo-Khattar, 1985) and prostaglandin release (Thulesius *et al.*, 1987).

In the present study we have shown that the anti-inflammatory glucocorticoids have an inhibitory action on ureteral smooth muscle similar to that of NSAIDs. This effect was shown to be dose-dependent and complete inhibition of motility with dexamethasone at a high concentration of 10^{-4} M was seen after approximately 24 min which is comparable to that observed for dexamethasone inhibition of prostaglandin release in rabbit coronary microvessel endothelial cells (Rosenbaum *et al.*, 1986). This time lag is indicative of an intranuclear site of action and pretreatment of ureteral strips with cycloheximide, an inhibitor of protein synthesis (Vazquez, 1974) prevented the action of dexamethasone, consistent with the suggestion that steroid action on the ureter is dependent upon the generation of lipocortin, the inhibitory phospholipase modulatory protein (Flower & Blackwell, 1979). The simulation of lipocortin release by steroids has been shown to occur in a number of tissues including rat macrophages (Blackwell *et al.*, 1980), rabbit neutrophils (Hirata *et al.*, 1979) rat renomedullary interstitial cells (Russo-Marie & Duval, 1982) and rabbit coronary microvessel endothelial cells (Rosenbaum *et al.*, 1986). Both the release of arachidonic acid (Hong & Levine, 1976) and histamine (Grosman & Jensen, 1984) are inhibited by glucocorticoids. We have recently reported ultrastructural evidence for the abundant presence of mast cells in the ureter (Al-Ugaily *et al.*, 1988) and although there is no sound explanation as to their possible normal physiological function, it is likely that prostaglandin and histamine release could in part be responsible for the hypermotility and pain associated with ureteral obstruction. Consequently one may speculate that the

Table 1 EC_{50} and relative potency of two non-steroidal anti-inflammatory drugs and three corticosteroids

	EC_{50}	Relative potency	n
Indomethacin	$2.1 \pm 2.0 \times 10^{-6}$ M	1	15
Diclofenac Na	$1.1 \pm 0.5 \times 10^{-6}$ M	1.9	10
Dexamethasone	$2.8 \pm 0.6 \times 10^{-6}$ M	0.75	8
Methyl prednisolone	$1.2 \pm 0.4 \times 10^{-5}$ M	0.18	8
Hydrocortisone	$3.8 \times 1.3 \times 10^{-4}$ M	0.008	6

Mean values $\pm$ s.e. are given; n = number of experiments.

additive effects of prostaglandin synthesis inhibition and suppression of histamine release from mast cells by glucocorticoids would make them valuable agents in the treatment of ureteral colic.

The rank order of potency of the steroids was found to be dexamethasone > methylprednisolone > hydrocortisone, which is in agreement with data from the literature (Haynes & Murad, 1985). Furthermore, dexamethasone was found to be similar in potency to the non-steroidal anti-inflammatory agents.

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