Influence of the nervous system on experimentally induced urethral inflammation

L. Nordling, H. Liedberg, P. Ekman and T. Lundeberg

1Department of Urology, Karolinska Hospital, Stockholm (Sweden) and 2 Department of Physiology II, Karolinska Institute, Stockholm (Sweden)

(Received 19 January 1990; Revised version received 13 March 1990; Accepted 16 March 1990)

Key words: Neurogenic inflammation; Urethra; Capsaicin; Sympathectomy; Morphine

We have studied the contribution of the nervous system on experimentally induced urethral inflammation in the rat. Urethral inflammation was induced by inserting latex strips into the urethra. The effects of different experimental procedures was assessed by using a 4-graded inflammation scale based on the histological findings. Attenuation of urethral inflammation was produced by administration of capsaicin at birth. A more complete attenuation was produced by sympathectomy prior to application of the latex strip. Urethral inflammation was also severe in the spontaneous hypertensive rat. Injection of morphine into the third ventricle of the brain significantly reduced the experimentally induced urethral inflammation. These data taken together indicate that the sensory and postganglionic innervation of the urethral mucosa as well as the central nervous system is critically involved in the inflammatory reaction of the urethra following exposure to latex strips.

Indwelling urethral catheters are often necessary in medical care. Being a foreign body the catheter may cause pain and inflammation within the urethra. Recently it has been suggested that the sensory and autonomic nervous systems may contribute to the inflammatory process [6]. The release of the peptide neurotransmitter substance P from the peripheral terminals of nociceptive afferent neurons and the release of catecholamines and peptides from postganglionic sympathetic efferent neurons produce physiologic changes associated with inflammation [8]. The activation of central neural circuits elicits similar physiologic changes, and lesions of the peripheral and central nervous systems are associated with alteration in activity of inflammation. The impact of these neurogenic mechanisms on the urethral inflammatory reaction has not yet been evaluated. The aim of the present study was to relate the neurogenical inflammatory reaction to the severity of catheter-induced urethral inflammation in an experimental rat model.

The study was carried out on 90 female albino rats (Sprague-Dawley, weight 180-260 g). The rats were anaesthetised with chloralhydrate (0.4 g/kg) and positioned su-
pine with the legs extended. A lower midline incision was made and a cystotomy performed. Strips (1 mm wide) of the surface of the midsection of the latex catheter to be tested were inserted into the urethra as far as the external meatus. The bladder was closed around a catheter tip of similar material and the proximal end cut flush with the skin to which it was fixed with black silk.

The rats received strips of the same latex catheter brand and batch after different treatments. Ten rats underwent cystotomy only (group 1). Ten rats serving as controls were tested with latex strips (group 2). To study the influence of small-diameter, unmyelinated and thinly myelinated primary afferents to the severity of urethral inflammation the neurotoxin capsaicin was used. This procedure produces a significant depletion of substance P and calcitonin gene-related peptide in afferent nerves. Ten rats were given capsaicin (50 mg/kg, s.c.) in a vehicle of 50% dimethyl sulfoxide and 50% saline on neonatal day 2 and thereafter daily until they reached a weight of about 200 g. These rats were then given latex strips (groups 3). The importance of the sympathetic nervous system to the severity of urethral inflammation was evaluated by sympathectomy using guanethidine or reserpine. Guanethidine produces a depletion of catecholamines by destroying the sympathetic postganglionic neurons. Guanethidine (5 mg/day) was administered to 10 rats for 6 weeks before the latex strip was given (group 4). As guanethidine may activate the immune system, another catecholamine depletor, reserpine, was used. Reserpine (0.25 mg/kg/day) was given to 10 rats starting 2 days before administration of the latex strip (group 5). To further study the influence of the sympathetic nervous system, a strain of rats having abnormally high activity in the sympathetic nervous system (SHR) was used. Ten spontaneously hypertensive rats (SHR) were given the latex strip (group 6). The normotensive Wistar-Kyota rat (WKY), which derives from the same, pedigree strain was used as a control. The latex strip was administered to 10 normotensive rats (WKY) (group 7). The influence of the central nervous system on the severity of the urethral inflammation was assessed by injection of morphine into the third ventricle of the brain, thereby activating control systems within the central nervous system. These control systems act at the spinal level inhibiting the activity of spinal neurons. Ten rats received morphine (25 µg in 1 µl of vehicle into the third ventricle) every 6 h for 72 via a stereotactically placed, chronically implanted cannula (group 8). Ten rats received saline into the third ventricle every 6 h for 72 h via a stereotactically placed, chronically implanted cannula (group 9).

After 72 h of latex strip administration, the rats were perfused with 2.5% glutaraldehyde in a 0.1 M phosphate buffer and sacrificed. After perfusion cystourethrectomy was carried out and a segment 5 mm long was dissected out. The specimens were postfixed in a glutaraldehyde, osmicated (2% osmium tetroxide in phosphate buffer, 30 min), rinsed in buffer, dehydrated in alcohol and embedded in Agar 100. Semithin and thin longitudinal sections were cut on an LKB ultratome. The semithin sections were stained with Toluidine blue and used for light microscopy. The effect of the different experimental procedures on the rat urethra was assessed using a 4-graded scale: 1—no oedema or other inflammatory sign; 2—mild oedema and loss of surface epithelium; no inflammatory exudate; 3—mild oedema and loss of surface epithelium; no inflammatory exudate; 3—inflammatory infiltrate, epithelial
Fig. 1. The effect of different experimental procedures on the rat urethra assessed by a 4 graded scale: 1—no oedema or other inflammatory sign (upper left); 2—mild oedema and loss of surface epithelium (upper right); 3—inflammatory infiltrate, epithelial loss (lower left); 4—inflammatory infiltrate, epithelial loss, exudate and haemorrhage (lower right).

loss; 4—inflammatory infiltrate, epithelial loss, exudate and haemorrhage (Fig. 1). Classification of inflammation severity was done in a blind setting.

For statistical analyses of degree (1–4) of inflammation between the different groups the Mann–Whitney U-test was used, $P < 0.05$ was considered significant.

The results of the present study are summarized in Table I. In the rats undergoing cystotomy only, the degree of inflammation was minimal. In the rats to which the latex strips were applied the degree of inflammation was significantly increased. The contribution of the small-diameter unmyelinated afferents to urethral inflammation was evaluated in capsaicin treated rats. The severity of the urethral mucosal inflammation was significantly less in rats given capsaicin than in latex rats not given capsaicin. The contribution of sympathetic innervation of the urethra to urethral inflammation was evaluated in sympathectomized rats. Guanethidine sympathectomy, completed one month prior to treatment with latex strip, significantly decreased the severity of the mucosal inflammation when compared with latex rats.

A similar reduction in urethral mucosal inflammation was produced by daily treatment with reserpine beginning 2 days before operation and continuing through the
TABLE

THE EFFECT OF DIFFERENT EXPERIMENTAL PROCEDURES ON THE RAT URETHRA ASSESSED BY A 4 GRADED SCALE

1—no oedema or other inflammatory sign; 2—mild oedema and loss of surface epithelium; 3—inflammatory infiltrate, epithelial loss; 4—inflammatory infiltrate, epithelial loss, exudate and haemorrhage.

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental procedure</th>
<th>Sample size</th>
<th>Median</th>
<th>Min.</th>
<th>Max.</th>
<th>Rank sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cystotomy</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>Latex</td>
<td>10</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>Capsaicin + latex</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>Guanethidine + latex</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>Reserpine + latex</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>Latex (SHR)</td>
<td>10</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>36</td>
</tr>
<tr>
<td>7</td>
<td>Latex (WKY)</td>
<td>10</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td>8</td>
<td>Morphine + latex</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>9</td>
<td>Saline + latex</td>
<td>10</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>34</td>
</tr>
</tbody>
</table>

period of observation. As a further test the contribution of the sympathetic nervous system to urethral inflammation was evaluated using SHR rats. SHR rats have an increased activity of their sympathetic nervous system with respect to their parent, normotensive strain (WKY rats). Urethral inflammation in the SHR rats was severer than in the WKY rats, however, not significant.

The contribution of the central nervous system to urethral inflammation was assessed in rats treated with intracerebroventricular morphine. Urethral inflammation of the rats treated with intracerebroventricular morphine was significantly less as compared to the rats treated with saline. It was also significantly less than the urethral inflammation seen in the rats only subjected to latex. No significant difference between latex rats and those injected with saline in the cerebral ventricles was found.

Previous studies have shown that indwelling urinary catheters cause an inflammatory response with a micropurulent discharge in the urethra [1]. It has been suggested that there is an interaction between tissue toxic catheters and reduced local blood circulation in the induction of urethral strictures [4, 12–14]. Talja and collaborators have shown that the tissue toxic chemicals are dissolved from latex catheters [13] and that the wash out of the toxic substances may be lowered under the reduced local blood circulation [14].

The present study shows that the degree of experimentally induced urethral inflammation in the rat also reflects changes in the activity of both afferent and efferent components of the nervous system. Reduction of urethral inflammation was produced by administration of capsaicin at birth and sympathectomy prior to inducing urethral inflammation by application of a latex strip.
Lewis [11] showed that primary afferent nociceptors contribute to the production of inflammation itself and this so-called neurogenic inflammation has also been demonstrated in experimentally induced arthritis [3, 8]. Peripheral nerve section attenuates the severity of arthritis, and capsaicin treatment, which destroys or inactivates C-fibre nociceptive afferents, reduces swelling and hyperalgesia of arthritic joints [3,7]. Ankle joints, which are more severely affected in arthritis, have lower nociceptive thresholds and higher substance P content than the less affected knee joints suggesting that they have a higher innervation density [8]. If substance P, a proposed mediator of C-fibre neurogenic inflammation, is infused into the knee joint of experimentally induced arthritis rats, it greatly increases the inflammation and joint destruction [8]. In accordance with our findings it is likely that the 'neurogenic inflammation' may contribute to the inflammatory reaction secondary to urethral catheters. Catheterization may induce pain, mechanical pressure and result in the release of toxic substances from the catheter. All these stimuli can each or together trigger the release of neurogenic substances into the urethral mucosa.

Recent studies have shown that the peripheral limb of the sympathetic nervous system is involved in arthritis [5, 6]. It has been found that sympathectomy markedly reduced inflammation and joint injury in an experimental arthritis model [9]. It is important to note the β2-blockade was effective even if administered after the onset of clinically apparent arthritis. In patients with rheumatoid arthritis, treatment with the β-blocker propranolol, and regional sympathetic block with guanethidine have been found to reduce pain [10].

The similarities of the results obtained in the present study on urethral inflammation and arthritis indicate that local anaesthetics and β2-antagonist should be tried out in the treatment of catheter induced inflammation.

This work was supported with grants from Maud and Birger Gustavssons Stiftelse and Tore Nilssons Stiftelse.