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Effects of spasmolytic and/or non-steroidal antiinflammatory drugs on muscle hyperalgesia of ureteral origin in rats

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Abstract

Rats with artificial calculosis of one ureter develop hyperalgesia in the ipsilateral oblique musculature as evidenced by decreased vocalization threshold to electrical muscle stimulation lasting over a week. The aim of the study was to evaluate the effect on this hyperalgesia of spasmolytic anticholinergic and/or non-steroidal antiinflammatory drugs, common therapies for colic pain in humans. Rats implanted with a unilateral ureteral stone were treated for 10 days with: (1) saline; (2) hyoscine-*N*-butylbromide (15 mg/kg/day i.p.); (3) ketoprofen (15 mg/kg/day); or (4) hyoscine-*N*-butylbromide + ketoprofen (15 + 15 mg/kg/day). Oblique muscle vocalization thresholds were measured daily for 3 days before and 10 days after operation. Ipsilateral thresholds decreased significantly after stone implantation on: (1) seven days (max. 32%) for saline; (2) one day (max. 20%) for hyoscine-*N*-butylbromide; (3) one day (max. 18%) for ketoprofen, but did not change significantly for hyoscine-*N*-butylbromide + ketoprofen. These results indicate a protective effect against muscle hyperalgesia of ureteral origin by spasmolytic and antiinflammatory drugs, maximal when the two treatments are combined.

Keywords: Spasmolytic and/or non-steroidal antiinflammatory treatment; Ureteral calculosis, artificial; Muscle hyperalgesia, referred; (Rat)

1. Introduction

The phenomenon of muscular hyperalgesia in areas of referred pain from renal/ureteral calculosis is well-known and documented in clinics (Refs. in Ansell and Gee, 1990). Previous studies by this group have shown that patients with calculosis of the upper urinary tract develop hypersensitivity in the ipsilateral obliquus externus muscle (lumbar region, metamere L1). This hypersensitivity, revealed by a decrease in pain threshold to electrical and pressure muscle stimulation, appears soon after the first painful episodes and is accentuated by repetition of the colics (Vecchiet et al., 1989, 1990). It long outlasts the pain and may persist, although to a lesser extent, even after elimination of the stone (Giamberardino et al., 1994; Vecchiet et al., 1992).

Many clinical studies have investigated the effects of various pharmacological agents on the spontaneous painful symptomatology of urinary calculosis (Lundstam et al., 1982; Oosterlinck et al., 1990; Sanahuja et al., 1990; Youssef and Hanafi, 1989). In contrast, no information is available about the possible effects of these same treatments on the hyperalgesia, particularly to prevent and/or reduce its extent and duration. A clinical trial addressing this issue is problematic to design. Firstly it is difficult to have homogeneous populations of patients (exactly the same position of stone in the urinary tract, same number of colics, etc., factors known to influence site and degree of referred hyperalgesia) (Vecchiet et al., 1990). Secondly it is hard, for ethical reasons, to set up a proper control group, i.e., patients with calculosis of the same characteristics as the drug-treated groups but who receive no treatment for their pain.

This matter can however be approached more easily under standardized conditions in animal experiments. Our group has set up an animal model of referred muscle hyperalgesia from artificial calculosis of the upper urinary tract (Giamberardino et al., 1990, 1993a).

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Similarly to what is observed in patients, rats implanted with a unilateral ureteral stone develop hypersensitivity of the ipsilateral obliquus externus muscle, as shown by a decrease in vocalization threshold to electrical muscle stimulation. The decrease appears soon after implantation, is most marked on the first 4 days and then tends to diminish (5th–7th day) although lasting up to the 10th day. With this animal model the aim of the present study was therefore to evaluate and compare the effects on the muscle hyperalgesia of chronic treatments with spasmolytic-anticholinergic and/or non-steroidal antiinflammatory (NSAIDs) drugs, which are common therapies in patients with colics.

Part of the results reported in this paper have been published in abstract form (Giamberardino et al., 1993b).

2. Materials and methods

A total of 46 female Sprague-Dawley rats (weighing 200–260 g) were used for the study. The experimental protocol was approved by the University Animal Care and Use Committee and adhered to the guidelines of the International Association for the Study of Pain (Zimmermann, 1983).

2.1. Normal rats – preliminary experiment

Six animals were implanted under general anesthesia (pentobarbital i.p., 60 mg/kg) with bipolar wire electrodes in the obliquus externus muscle of one side (lumbar region, metamere L1). The isolated wires were passed under the skin towards the skull where connectors were fixed into the parietal bones by means of small screws and dental cement, a procedure allowing electrical stimulation in the freely moving animals for vocalization threshold measurement (Giamberardino et al., 1990,1993a).

Starting on the third day after muscle implantation, all animals were given chronic treatment with ketoprofen (15 mg/kg in three i.p. injections per day at 9.30 a.m., 2 p.m., 7.30 p.m.) for 10 days.

Vocalization thresholds to muscle stimulation were measured daily in each animal for 3 days before and 10 days after the start of treatment. Measurement was always at 9 a.m., that is, 30 min before the morning injection and therefore outside the phase of action of the drug (the time interval between injections was purposely kept longer during the night).

2.2. Stone-implanted rats – main experiment

Forty animals underwent electrode implantation of the obliquus externus muscle of both sides. For 3 days after the operation vocalization thresholds to electrical stimulation were measured daily for both muscles, at 9.00 a.m.

On the third day, soon after the measurement, the rats underwent a second operation under general anesthesia. Via a vertical suprapubic incision an artificial stone was formed in the upper third of the left ureter by injecting 0.02 ml of dental cement (while still fluid) in the lumen using a syringe with a 0.4 mm diameter needle (Giamberardino et al., 1990,1993a).

The rats were then randomly assigned to four groups of ten each, to undergo different chronic drug treatments for 10 days, starting about 5 h after the beginning of stone implantation. The groups were as follows: group 1: saline; group 2: spasmolytic-anticholinergic (hyoscine-*N*-butylbromide, 15 mg/kg/day); group 3: non-steroidal antiinflammatory (NSAID, ketoprofen, 15 mg/kg/day); group 4: spasmolytic-anticholinergic + non-steroidal antiinflammatory (hyoscine *N*-butylbromide + ketoprofen, 15 + 15 mg/kg/day). In groups 2–4 the total daily amount of drug was subdivided into 3 i.p. injections (at 9.30 a.m., 2 p.m., 7.30 p.m.). Group 1 animals received injections of equivalent amounts of saline following the same schedule.

Vocalization thresholds to electrical stimulation of the obliquus externus muscle of both sides were remeasured daily in all animals for the whole 10-day period of treatment, always at 9.00 a.m.

2.3. Post-mortem

At the end of the study (10th day after ureteral intervention) all stone-implanted animals of the four groups were killed via an overdose of pentobarbital and post-mortems were done for evaluation of urinary tract condition.

2.4. Electrical stimulation technique

250 ms trains of 1 ms square wave impulses (frequency 200/s) were used, delivered automatically every 2 s by a constant current stimulator (Lace Electronics device). Vocalization thresholds were measured according to the limit method. The intensity was increased in 0.3 mA steps until vocalization occurred, was then decreased in 0.1 mA steps until it disappeared and then re-increased at the latter rate until vocalization returned, and the corresponding values were noted in mA. The threshold was calculated as the mean of these values (Giamberardino et al., 1990, 1993a).

2.5. Statistical analysis

Means ± standard errors of the mean (S.E.M.) of vocalization thresholds to electrical stimulation were

calculated for each day for both normal and stone-implanted rats. One-way analysis of variance (ANOVA) was used to compare pre- and post-operative values in each group of animals. The Wilcoxon sum rank test was applied to compare the results of different drug treatments with those of saline treatment. Significance level was assessed at $P < 0.05$.

3. Results

3.1. Normal rats

Table 1 shows vocalization thresholds to electrical stimulation of the obliquus externus muscle of one side in the six normal rats for 3 days before and 10 days during chronic treatment with ketoprofen. No significant difference was found between pre- and post-treatment values.

3.2. Stone-implanted rats

Fig. 1A shows thresholds of the muscle ipsilateral to the implanted ureter for 3 days before and 10 days after stone implantation (and start of pharmacological treatment) in the four groups of rats. The maximum threshold decrease after treatment was: (1) 32% (day 2) for the saline group; (2) 20% (day 1) for the spasmolytic group; (3) 18% (day 2) for the antiinflammatory group; and (4) 13% (day 2) for the spasmolytic + antiinflammatory group.

Fig. 1B shows thresholds of the muscle contralateral to the implanted ureter. The slight threshold decrease present in the saline group after treatment (max. 12% on day 2) was not observed in the three groups treated with drugs.

Thresholds of the ipsilateral muscle (Fig. 1A) apply to all ten rats per group. Thresholds of the contralateral muscle (Fig. 1B) refer to ten rats for the saline group, nine for the spasmolytic and antiinflammatory groups and eight for the spasmolytic + antiinflammatory group (muscular electrodes in the remaining rats broke off before completion of measurements).

The comparison between the different treatments and saline for the muscle ipsilateral to the implanted ureter showed a significant difference for: (a) spasmolytic vs. saline on days 3–4 ($P < 0.03$ and $P < 0.0007$) and 7–10 ($0.003 > P < 0.02$); (b) antiinflammatory vs. saline on days 4–5 ($P < 0.0004$ and $P < 0.004$) and 9–10 ($P < 0.009$ and $P < 0.004$); (c) spasmolytic + antiinflammatory vs. saline on days 3–10 ($0.00002 > P < 0.02$). The comparison for the contralateral muscle showed no significant difference for spasmolytic vs.

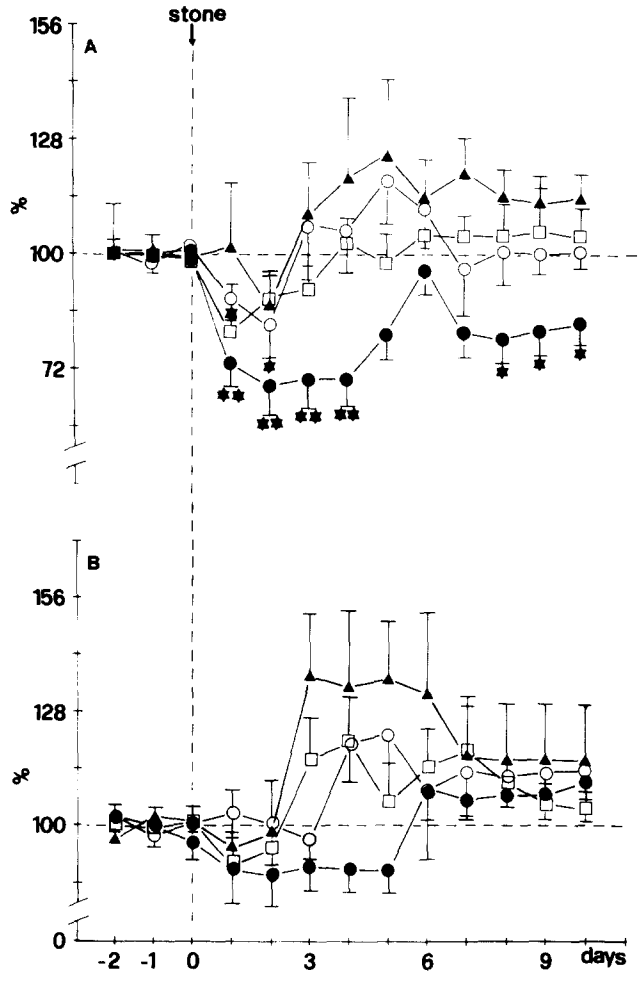

Fig. 1. Rats implanted with a stone in one ureter, chronically treated with saline (●), spasmolytic (□), antiinflammatory (○) and spasmolytic + antiinflammatory (▲) drugs respectively for 10 days (four groups of ten rats each). Vocalization thresholds to electrical stimulation of the obliquus externus muscle ipsilateral (A) and contralateral (B) to the implanted ureter. Day 0: day of stone implantation + start of pharmacological treatment. Post-implantation thresholds are expressed as a percentage of pre-implantation ones (means of thresholds recorded on days −2, −1, and 0) (means ± S.E.M.). Asterisks near S.E.M. bars of each curve refer to comparison between pre- and post-implantation values (one-way ANOVA). $^{*}P < 0.05$; $^{**}P < 0.01$.

Table 1
Vocalization thresholds to electrical stimulation of the obliquus externus muscle in six normal rats before (days −2, −1 and 0) and during (days 1–10) chronic treatment with ketoprofen (mA, mean ± S.E.M.)

Days	−2	−1	0	1	2	3	4	5	6	7	8	9	10
Mean	3.79	3.60	3.61	3.71	3.69	4.01	3.87	3.96	3.72	3.96	3.72	3.70	3.80
S.E.M.	0.33	0.44	0.55	0.43	0.55	0.43	0.44	0.48	0.46	0.42	0.35	0.36	0.37

saline, a significant difference for antiinflammatory vs. saline on day 4 ($P < 0.02$) and for spasmolytic + antiinflammatory vs. saline on days 3–5 ($0.04 > P < 0.02$).

3.3. Post-mortem

In each group, two main patterns were observed in the urinary tract: (1) stone no longer detectable (incidence of 20, 30, 40 and 10% in the spasmolytic, antiinflammatory, spasmolytic + antiinflammatory and saline groups, respectively); (2) stone still present either in the implant position or displaced in a lower ureteral position, with or without signs of spontaneous occlusion (dilated ureter proximal to the stone, increased kidney dimension).

For each of the four groups, the progress of thresholds only of rats with pattern 2) (i.e. still presenting the stone) was exactly comparable to that of all animals' thresholds shown in Fig. 1.

4. Discussion

Rats with an artificial stone implanted in one ureter, and chronically treated with saline, showed a decrease in vocalization thresholds to electrical stimulation of the ipsilateral lumbar muscles (hyperalgesia) which was exactly comparable in extent and duration to that of calculosis rats with no treatment (Giamberardino et al., 1990).

Stone-implanted rats chronically given spasmolytic-anticholinergic or non-steroidal antiinflammatory drugs, in contrast, showed a reduction of both extent and duration of the ipsilateral muscle threshold decrease with maximum efficacy when the two treatments were combined. The rats also showed a reduction of the slight, although not significant, threshold decrease that appears in the contralateral muscle.

In the present study the doses of spasmolytic and antiinflammatory drugs were the highest which, administered chronically to normal animals, proved not to directly affect vocalization thresholds to electrical muscle stimulation (for spasmolytics, this had been established in a previous study, for antiinflammatories it was shown in the present study) (Giamberardino et al., 1993a). Since any direct influence of the drugs on muscle sensitivity has to be excluded, the reduction of muscle hyperalgesia in calculosis animals must therefore be mediated through a decrease in the abnormal algogenic input from the ureter and/or in its effect on central neurons (MacKenzie, 1909). This can certainly occur via promotion of the visceral focus extinction since post-mortem showed an increased percentage of stone elimination in treated rats. However, the reduction of referred hyperalgesia was clearly evident also in rats still presenting the stone in the ureter at the end of the treatment(s). Therefore the explanation of the phenomenon must be searched for on the basis of the pathophysiology of pain from ureteral calculosis on one hand and on the mechanism of action of the drugs used on the other.

As well known, colic pain in ureteral calculosis derives mainly from acute pelvis dilatation due to impact of the stone in the ureteral lumen (Ansell and Gee, 1990). Ureteric obstruction furthermore causes increased synthesis and release of prostaglandins which in turn both increase glomerular filtration and renal pelvic pressure and sensitize nociceptors locally (Refs. in Braga, 1990). An important algogenic role is also played by ureteral wall contractility since there is clear experimental evidence that hypermotility takes place above the obstacle (Rose and Gillenwater, 1973; Thulesius et al., 1989). The efficacy of ketoprofen certainly relies upon its peripheral action as a potent prostaglandin synthetase inhibitor, resulting in a reduced algogenic input from the ureter, but also on its well-recognized central analgesic action (Braga, 1990; Willer et al., 1989). Clinical studies in fact clearly evidence that the extent of referred hyperalgesia is strictly dependent on the degree of the pain experienced (Vecchiet et al., 1990). The efficacy of hyoscine-*N*-butylbromide, in contrast, can only be due to a reduction of ureteral hypermotility, since no direct analgesic property of the compound has been documented at the dose used (Giamberardino et al., 1993a).

In conclusion the results of the study showed that prolonged treatment with spasmolytic and antiinflammatory drugs is able to prevent to a large extent the referred hyperalgesia subsequent to ureteral calculosis and that the efficacy is enhanced by the combined use of the drugs.

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