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Effect of dexamethasone on cyclophosphamide-induced cystitis in rats: lack of relation with bradykinin B_1 receptor-mediated motor responses

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Abstract

We investigated the role of bradykinin B_1 receptors in inducing urinary bladder contraction and maintaining bladder compliance in anaesthetized rats following cyclophosphamide-induced bladder inflammation and the influence of dexamethasone treatment on these responses. In the group treated with cyclophosphamide the amplitude of the contraction induced by the selective bradykinin B_1 receptor agonist des-Arg^9-bradykinin was larger than that in controls and dexamethasone prevented the up-regulation of this response induced by inflammation. The specific binding of [^{3}H]des-Arg^{10}-kallidin to bladder membranes was only detected in cyclophosphamide-treated rats: this binding was prevented by dexamethasone pretreatment. The bladder contraction induced by des-Arg^9-bradykinin in cyclophosphamide-treated rats was antagonized by the bradykinin B_1 receptor antagonist des-Arg^9-D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin (des-Arg^{10}-Hoe 140). Cyclophosphamide treatment increased the bladder weight and dexamethasone reversed this effect. Bladder compliance was decreased in the bladder inflammation group and this effect was partially reversed by dexamethasone pretreatment. Neither des-Arg^{10}-Hoe 140 nor the combined administration of des-Arg^{10}Hoe 140 and the selective bradykinin B_2 receptor antagonist D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin (Hoe 140) affected bladder compliance, thus excluding a role of kinins in the maintenance of bladder tone during inflammation. These results indicate that: (1) dexamethasone pretreatment ameliorates cyclophosphamide-induced bladder inflammation; (2) dexamethasone pretreatment prevents cyclophosphamide-induced up-regulation of bradykinin B_1 receptors; (3) kinins do not contribute to the increased vesical tone during inflammation.

Keywords: Bladder compliance; Bradykinin B_1 receptor binding; Bradykinin B_2 receptor binding; Des-Arg^{10}-Hoe 140; Hoe 140; Inflammation; Kinin; Urinary bladder

1. Introduction

Kinins are small pro-inflammatory peptides (8–10 amino acids) produced by the proteolytic cleavage of larger precursors termed kininogens. This process occurs at sites of inflammation or tissue injury, and therefore it is believed that kinins play an active role during inflammation (Bhoola et al., 1992). Two different receptors, initially identified through pharmacological criteria, mediate the biological effects of kinins. These receptors, termed bradykinin B_1 and B_2, can be distinguished on the basis of the agonist activity of naturally occurring kinins in various isolated preparations. Bradykinin B_2 receptors are selectively stimulated by the plasma kinin called bradykinin and its tissue counterpart kallidin, whereas selective agonists for bradykinin B_1 receptors are metabolites that lack the C-terminal arginine, namely, des-Arg^9-bradykinin and des-Arg^{10}-kallidin, respectively (Regoli and Barabé, 1980). This original classification has been fully confirmed in cloning experiments in which two different molecular entities corresponding to the bradykinin B_1 and B_2 receptors were identified (Hess, 1997). Beyond their pharmacological properties, the biology of the two receptors differs in other aspects: one of the most striking differences is that, with a few exceptions (Marceau et al., 1998), bradykinin B_1 receptors are synthesized de novo following inflammation or tissue injury whereas bradykinin B_2 receptors are constitutively expressed in many cell types (Marceau, 1995). The rat urinary bladder does not make exception to

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this rule: following experimental inflammation, bradykinin B_1 receptor-mediated contractile responses have been detected (Marceau et al., 1980). Steroidal anti-inflammatory drugs like dexamethasone reduce several components of the inflammatory response (Flower, 1988), including the expression of bradykinin B_1 receptors (Marceau, 1995). Given the potential importance of kinins in determining symptoms of cystitis (Maggi, 1997; Jaggar et al., 1998; Rosamilia et al., 1998), this study was aimed at defining the role of bradykinin B_1 receptors in the regulation of bladder smooth muscle tone during bladder inflammation. For this purpose, we used the model of cyclophosphamide-induced haemorrhagic cystitis (Maggi et al., 1992), an inflammatory condition in which bradykinin B_1 receptor-mediated contractile responses in isolated bladder strips are up-regulated (Meini et al., 1998). In this model of cystitis, we assessed the effect of dexamethasone on the up-regulation of the bradykinin B_1 receptor-mediated contractile responses in vivo and on the expression of bradykinin B_1 receptors in the bladder. Furthermore, we determined the effect of cyclophosphamide pretreatment on bladder compliance during saline infusion. Finally, the effect of dexamethasone on bladder compliance was compared to the effect of the selective bradykinin B_1 receptor antagonist des-Arg^9-D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8] bradykinin (des-Arg^{10}-Hoe 140) (Wirth et al., 1991a) alone or in combination with the selective bradykinin B_2 receptor antagonist D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin (Hoe 140) (Wirth et al., 1991b).

2. Material and methods

2.1. In vivo studies

Male Wistar rats (Charles River, Calco, Italy) weighing 330–380 g were treated with cyclophosphamide (150 mg/kg, i.p., 48 h before the experiments) or saline. Dexamethasone (1 mg/kg, s.c.) or its vehicle (saline) was administered 49, 41, 25, 17 and 1 h before the experiments. Experiments were carried out under urethane anaesthesia (1.2 g/kg, s.c.), in animals subjected to the acute (1–2 h before), bilateral removal of the pelvic ganglia. Pelvic ganglia were surgically ablated as previously described (Lecci et al., 1994). After ureter ligation, the urinary bladder was exposed and a polyethylene catheter (PE 90, I.D. 0.86 O.D. 1.27 mm) was inserted and secured to the proximal urethra for intravesical pressure recording. The free end of the catheter was connected to a pressure transducer and intravesical pressure was recorded by means of a polygraph. The left jugular vein was cannulated for the i.v. administration of compounds. The dose–response curve for des-Arg^9-bradykinin (0.5 log unit dose increase at 30 min intervals, 1–300 nmol/kg i.v.) was recorded under isovolumetric (0.5 ml) conditions. After the dose–response curve was recorded, the contractile effect of the selective tachykinin NK_2 receptor agonist [βAla^8]neurokinin A-(4–10) (3 nmol/kg, i.v.) was also evaluated. The whole experiment lasted about 4 h. The effect of the selective bradykinin B_1 receptor antagonist des-Arg^{10}-Hoe 140 was evaluated on the motor response induced by des-Arg^9-bradykinin in ganglionectomized, cyclophosphamide-treated rats. After a 90-min stabilization period, des-Arg^9-bradykinin (100 nmol/kg, i.v.) was administered, and the bradykinin B_1 receptor agonist challenge was repeated three times at 1-h interval. The whole experiment lasted about 4 h. Des-Arg^{10}-Hoe 140 (1 μmol/kg, i.v.) or its vehicle (saline) was injected 10 min before the second agonist challenge. The effect of the acute dexamethasone treatment (1 mg/kg, i.v.) on the contractile response induced by des-Arg^9-bradykinin (100 nmol/kg, i.v.), [βAla^8]neurokinin A-(4–10) (3 nmol/kg, i.v.), or endotoxin (2 mg/kg, i.v.) in control and in cyclophosphamide-treated rats was evaluated under similar experimental conditions. Briefly, the bladder contraction induced by des-Arg^9-bradykinin or [βAla^8]neurokinin A-(4–10) was determined after a 30-min stabilization period (about 120 min after the surgical procedures for the intravesical pressure recordings). One hour after the first challenge with the agonists, dexamethasone or saline was administered and the effect of the bradykinin B_1 or tachykinin NK_2 receptor agonist was assessed again 1 h later. These experiments lasted about 150 min. The effect of dexamethasone on endotoxin-induced bladder contraction was assessed 1 h after the coadministration of endotoxin with dexamethasone or vehicle.

Bladder compliance was measured during slow saline infusion (50 μl/min) in animals subjected to acute ablation of the pelvic ganglia. The effect of Hoe 140 (100 nmol/kg, i.v.) or des-Arg^{10}-Hoe 140 (1 μmol/kg, i.v.) on bladder compliance was studied after the determination of basal compliance 55 min before the antagonist administration and 60 min before a second measurement of compliance. The intravesical infusion of saline for the bladder compliance measurement lasted 10 min.

In separate groups of animals, which underwent cyclophosphamide and dexamethasone treatment, the urinary bladder wet weight was also recorded. In these animals the urinary bladders were excised after death, emptied of urine and weighed.

2.2. Binding studies

Rats were treated with cyclophosphamide (150 mg/kg, i.p., 48 h before the experiments) or saline. Dexamethasone (1 mg/kg, s.c.) or its vehicle (saline) was administered 49, 41, 25, 17 and 1 h before the animals were killed. The animals were stunned and bled. The bladders were removed, dissected free of connective tissue, longitudinally opened, weighed and placed in *n*-tris[hydroxymethyl] methyl-2-aminoethanesulphonic acid buffer (TES 10 mM, pH 7.4, at 4°C) containing a cocktail of peptidase in-

hibitors: 1,10 phenanthroline (1 mM), ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (1 mM), captopril, leupeptin, soybean trypsin inhibitor, DL-2-mercaptomethyl-3-guanidoethylthiopropanoic acid (1 μM each), chymostatin (3.3 μM), phenylmethyl-sulphonyl fluoride (0.1 mM), and bacitracin (140 μg/ml). The bladders were chopped (McIlwain Tissue Chopper, The Mickle Laboratory Engineering) and homogenized on ice in 30 volumes of buffer with an Ultra-Turrax T25 (Janke & Kunkel, IKA-Labor Technick) set at 24 000 rpm. The homogenate was centrifuged at $1000 \times g$ for 10 min to remove cellular debris. The supernatant was collected and the cellular debris was resuspended, homogenized, and centrifuged as above. The supernatants were mixed and centrifuged at 17 000 rpm (4°C) for 45 min. The pellet was resuspended in binding buffer and frozen immediately by immersion in liquid nitrogen, and then stored at −80°C until use.

The protein concentration was determined by the method of Bradford (1976) using a Bio-Rad kit and bovine serum albumin as reference standard. Immediately prior to use, frozen membrane aliquots were thawed in binding buffer (see below) and mixed to give a homogeneous membrane suspension. The buffer used for binding experiments was TES (10 mM, pH 7.4) containing 1,10 phenanthroline (1 mM), bacitracin (140 μg/ml), and bovine serum albumin (1 g/l). The radioligands [^{3}H]des-Arg^{10}-kallidin and [^{3}H]bradykinin, which bind to bradykinin B_1 and B_2 receptors, respectively, were used at the concentration of 0.5 nM. Non-specific binding was defined as the amount of labelled ligand bound in the presence of 1 μM of des-Arg^{10}-kallidin and bradykinin, respectively. The binding assay was performed in quadruplicate in polypropylene tubes in a final volume of 0.5 ml. An incubation time of 90 min at 4°C was used.

All incubations were terminated by rapid filtration of the assay mixture through Whatman GF/B glass-fibre filtermats that had been presoaked for at least 2 h in polyethylenimine 0.6%, using a Brandel 48 cell harvester. The tubes and filters were then washed four times with 3-ml aliquots of Tris buffer (50 mM, pH 7.4, 4°C). Filters were soaked in CytoScint scintillation fluid (ICN Biomedicals) overnight, and bound radioactivity was counted in a β-scintillation counter (2200 CA, Packard).

2.3. Drugs

Cyclophosphamide monohydrate and dexamethasone phosphate disodium salt were purchased from Sigma (St. Louis, MO, USA). Endotoxin from *E. coli* serotype 0111: B4 was bought from Difco (Detroit, MI, USA). [βAla^8]neurokinin A-(4–10), Hoe 140 and des-Arg^{10}-Hoe 140 were synthesized in the Chemistry Research Department of Menarini Ricerche, Florence, Italy. Des-Arg^9-bradykinin was provided by Peninsula (Merseyside, UK). [^{3}H]des-Arg^{10}-kallidin (specific activity 91 Ci/mmol), and [^{3}H]bradykinin (specific activity 90 Ci/mmol) were provided by Du Pont NEN (Hertfordshire, UK). Des-Arg^{10}-kallidin was obtained from Peninsula (Merseyside, UK) and BK from Neosystem (Strasbourg, France). Leupeptin was obtained from Boehringer Mannheim (Germany) and DL-2-mercaptomethyl-3-guanidoethylthiopropanoic acid from Calbiochem (La Jolla, CA, USA). GF/B glass-fibre filtermats were provided by Brandel (Semat, St. Albans, Herts, UK). All other materials and reagents were obtained from Sigma.

2.4. Statistics

One-, two- or three-way analysis of variance followed by Fisher LSD (least significant difference) test was used for data analysis.

3. Results

3.1. In vivo studies

Cyclophosphamide treatment (150 mg/kg, i.p., 48 h before) induced cystitis, as indicated by the large increase in bladder weight compared to that of controls. Dexamethasone pretreatment (1 mg/kg, s.c., 49, 41, 25, 17 and 1 h before) did not significantly affect the bladder weight on its own, but blocked the increase in bladder weight induced by cyclophosphamide (Table 1).

In order to see whether cyclophosphamide treatment induces changes in the visco-elastic properties of the bladder wall, urinary bladder compliance was measured in animals subjected to bilateral ablation of the pelvic ganglia. This was done to eliminate reflex modulation of smooth muscle contractility. As shown in Fig. 1, cyclophosphamide-induced inflammation resulted in a dramatic decrease in bladder compliance. This effect was partially reversed by dexamethasone pretreatment, which had no effect on the bladder compliance in control animals (Fig. 1).

In control animals subjected to ablation of pelvic ganglia, administration of the selective bradykinin B_1 receptor agonist des-Arg^9-bradykinin (1–300 nmol/kg, i.v.) elicited

Table 1
Effect of dexamethasone (1 mg/kg, s.c., 49, 41, 25, 17 and 1 h before) or saline pretreatment on urinary bladder wet weight in control or cyclophosphamide-treated rats

Treatment	Bladder weight (mg)	
	Control	Cyclophosphamide
Saline	86 ± 7	154 ± 6[a]
Dexamethasone	76 ± 5	87 ± 4[b]

Each value represents the mean ± S.E.M. from 10 experiments. Fisher's LSD test: [a] $P < 0.01$ vs. saline–control; [b] $P < 0.01$ vs. saline-cyclophosphamide.

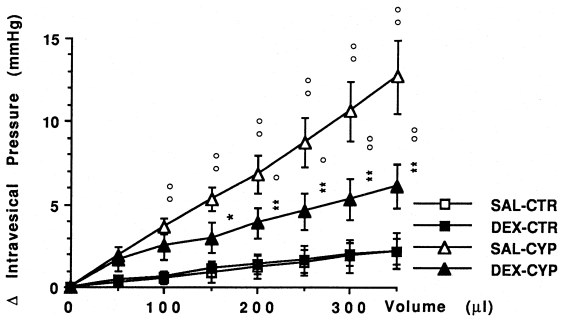

Fig. 1. Effect of dexamethasone (DEX) (1 mg/kg, s.c., 49, 41, 25, 17 and 1 h before) or saline (SAL) pretreatment on urinary bladder compliance in controls (CTR) or cyclophosphamide (CYP)-treated rats. Each point and bar represents the mean ± S.E.M. of eight experiments. Fisher's LSD test: $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. SAL–CYP, $^{\circ}P < 0.05$ and $^{\circ\circ}P < 0.01$ vs. SAL–CTR or DEX–CTR, $n = 8$ for each group.

a small (< 5 mm Hg) tonic urinary bladder contraction in both vehicle- and dexamethasone-pretreated animals (Fig. 2A). In cyclophosphamide-treated rats, this contractile response was greatly amplified (≥ 10 mm Hg). Dexamethasone pretreatment completely prevented the cyclophosphamide-induced up-regulation of the bladder contractile response to des-Arg^9-bradykinin (Fig. 2A). In contrast, the bladder contraction induced by the selective tachykinin NK_2 receptor agonist [βAla^8]neurokinin A-(4–10) (3 nmol/kg, i.v.) did not change after cyclophosphamide or dexamethasone pretreatment (Fig. 2B). The effect of acute administration of dexamethasone (1 mg/kg, i.v., 1 h before) was assessed after a control challenge with des-Arg^9-bradykinin (100 nmol/kg, i.v.) or [βAla^8]neurokinin A-(4–10) (3 nmol/kg, i.v.) in both control or cyclophosphamide-treated rats. In control animals, about 2 h after the surgical procedures for the intravesical pressure recordings, des-Arg^9-bradykinin elicited a small tonic contraction whose amplitude increased significantly 2 h after vehicle treatment (Table 2A). Dexamethasone had no effect on the des-Arg^9-bradykinin-induced bladder contraction but reduced the time-dependent up-regulation of this response. In cyclophosphamide-pretreated animals, the bladder contraction induced by des-Arg^9-bradykinin was larger than in controls but showed a similar time-dependent increase after vehicle administration (Table 2B). As in controls, dexamethasone had no effect on des-Arg^9-bradykinin-induced bladder contraction but blocked the time-dependent up-regulation of this response (Table 2B). In contrast, the bladder contraction induced by [βAla^8]neurokinin A-(4–10) did not show time-dependent variations either in controls or in cyclophosphamide-pretreated animals and dexamethasone did not affect this response (Table 2A and B). In controls, the bladder contraction induced by endotoxin (2 mg/kg, i.v., 1 h before) was prevented by dexamethasone coadministration (Table 2A). In cyclophosphamide-

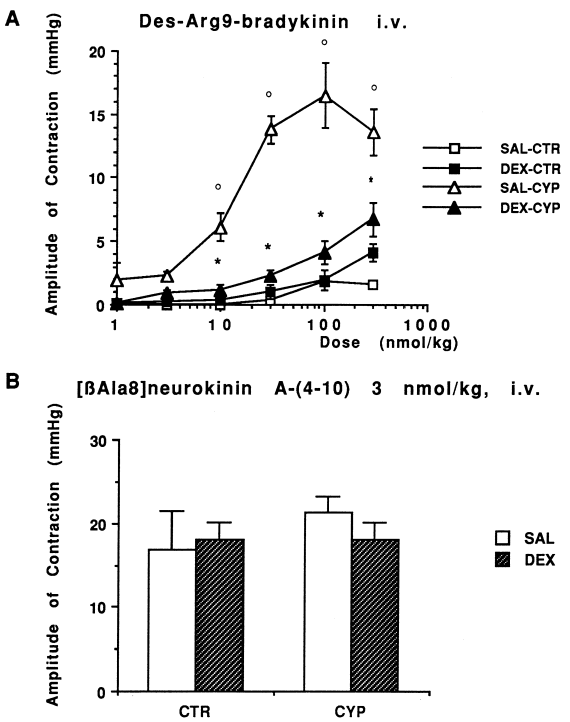

Fig. 2. Effect of dexamethasone (DEX) (1 mg/kg, s.c., 49, 41, 25, 17 and 1 h before) or saline (SAL) on the contractile effect induced by (A) des-Arg^9-bradykinin or (B) [βAla^8]neurokinin A-(4–10) in rats with cyclophosphamide (CYP)-induced urinary bladder inflammation or in control rats (CTR). Fisher's LSD test: $^{*}P < 0.01$ vs. SAL–CYP, $^{\circ}P < 0.01$ vs. SAL–CTR, $n = 8$ for each group.

Table 2
Effect of vehicle (saline) or acute dexamethasone treatment (1 mg/kg, i.v., 1 h before) on the local bladder contraction (after pelvic ganglionectomy) induced by des-Arg^9-bradykinin (100 nmol/kg, i.v.), [βAla^8]neurokinin A-(4–10) (3 nmol/kg, i.v.) or endotoxin (2 mg/kg, i.v.) in control or cyclophosphamide-pretreated rats

	Amplitude of the local bladder contraction (mm Hg)			
	Vehicle		Dexamethasone	
	Before	After	Before	After
(A) Control				
Des-Arg^9-bradykinin	2.2 ± 0.3	4.5 ± 0.4[a]	2.4 ± 0.4	3.6 ± 0.4
[βAla^8] neurokinin A-(4–10)	28.7 ± 3.5	27.4 ± 3.9	29.8 ± 3.3	26.9 ± 3.2
Endotoxin		4.5 ± 0.6		0.1 ± 0.2[c]
(B) Cyclophosphamide				
Des-Arg^9-bradykinin	15.7 ± 1.5	23.8 ± 3.0[b]	21.4 ± 3.2	22.7 ± 3.5
[βAla^8] neurokinin A-(4–10)	18.5 ± 3.1	15.5 ± 2.8	21.7 ± 1.9	19.7 ± 3.6
Endotoxin		1.1 ± 1.7		−0.7 ± 1.0

There was a 2-h interval between the two challenges with des-Arg^9-bradykinin or [βAla^8]neurokinin A-(4–10). Endotoxin was coadministered with dexamethasone or its vehicle. The bladder contraction induced by endotoxin was evaluated 1 h after drug administration. Each value represents the mean ± S.E.M. of seven to eight experiments. Fisher's LSD test: [a]$P < 0.05$ and [b]$P < 0.01$ vs. before; [c]$P < 0.01$ vs. vehicle.

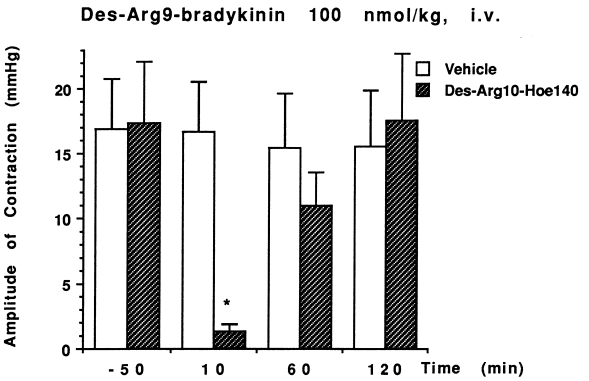

Fig. 3. Effect of des-Arg[10]-Hoe 140 (1 μmol/kg, i.v.) or its vehicle (saline) on des-Arg[9]-bradykinin (100 nmol/kg, i.v.)-induced bladder contraction in rats with bladder inflammation subjected to acute ablation of the pelvic ganglia. On the *X*-axis the time from des-Arg[10]-Hoe 140 administration is reported. Each column and bar represents the mean ± S.E.M. of five experiments. Fisher's LSD test: * $P < 0.01$ vs. vehicle.

pretreated rats endotoxin had no significant effect (Table 2B).

The effect of the selective bradykinin B_1 receptor antagonist des-Arg[10]-Hoe 140 (1 μmol/kg, i.v.) was tested against the bladder contraction induced by des-Arg[9]-bradykinin (100 nmol/kg, i.v.) at various times after the antagonist administration in cyclophosphamide-pretreated animals. The contractile response to des-Arg[9]-bradykinin did not vary significantly during the course of the experiment (Fig. 3). This is probably because about 3 h elapsed between the surgical procedure and the first challenge with des-Arg[9]-bradykinin, a time when the time-dependent up-regulation of bradykinin B_1 receptor-mediated contractile response reaches to a plateau (Lecci et al., 1998). At 10 min after its administration, des-Arg[10]-Hoe 140 almost abolished (92% inhibition) the bladder contraction induced by the bradykinin B_1 receptor agonist; however, at 60 min the effect of the antagonist was no longer significant (36% inhibition) and had completely disappeared at 120 min (Fig. 3).

To determine whether the decrease in bladder compliance observed in cyclophosphamide-treated animals involves tonic smooth muscle contraction elicited by endogenous kinins acting through bradykinin B_1 and/or B_2 receptors, the effect of des-Arg[10]-Hoe 140 (1 μmol/kg, i.v., 5 min before) on bladder compliance was assessed alone or in combination with the selective bradykinin B_2 receptor antagonist Hoe 140 (100 nmol/kg, i.v., 5 min before) after the induction of inflammation. Compliance measurements could be repeated at 1-h intervals without significant variations (Fig. 4A). Des-Arg[10]-Hoe 140 either alone (Fig. 4B) or in combination with Hoe 140 (Fig. 4C) did not affect bladder compliance in cyclophosphamide-treated rats.

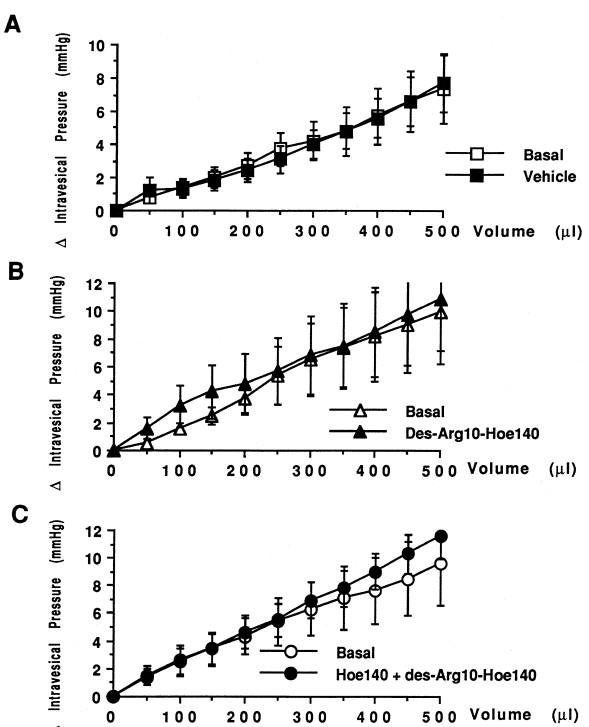

Fig. 4. Effect of vehicle (panel A) or des-Arg[10]-Hoe 140 (1 μmol/kg i.v., 5 min before) (panel B) or a mixture of des-Arg[10]-Hoe 140 (1 μmol/kg i.v., 5 min before) and Hoe 140 (100 nmol/kg, i.v., 5 min before) (panel C), on bladder compliance in the cyclophosphamide-treated group. Each point and bar represents the mean ± S.E.M. of six experiments. Bladder compliance was measured two times in each animal at 60-min intervals, before (basal) and 5 min after treatment.

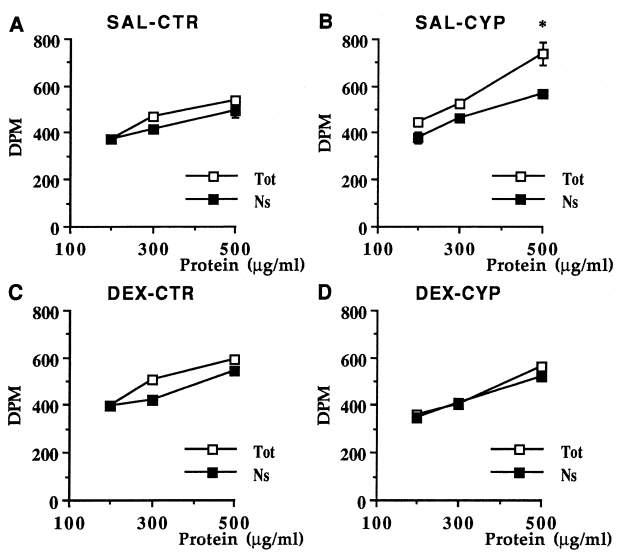

Fig. 5. Effect of saline (SAL, panels A and B) or dexamethasone (DEX, panels C and D) pretreatment in control (CTR, panels A and C) or cyclophosphamide-treated rats (CYP, panels B and D) on [^{3}H]des-Arg[10]-kallidin binding to urinary bladder membranes as a function of the protein content (μg). DPM, disintegrations per min; Tot, total binding (0.5 nM of [^{3}H]des-Arg[10]-kallidin; Ns, non-specific binding (0.5 nM of [^{3}H]des-Arg[10]-kallidin and 1 μM of des-Arg[10]-kallidin. Each point and bar represents the mean ± S.E.M. of four determinations. Fisher's LSD test: * $P < 0.01$ vs. Ns.

3.2. Binding studies

No specific binding of [^{3}H]des-Arg^{10}-kallidin (a radioligand for bradykinin B_1 receptors) could be detected in urinary bladder membranes from control animals pretreated with dexamethasone (1 mg/kg, s.c., 49, 41, 25, 17 and 1 h before death) or its vehicle, at any protein concentration investigated (Fig. 5A and C). In contrast, membranes from cyclophosphamide-treated animals displayed specific binding of [^{3}H]des-Arg^{10}-kallidin which was statistically significant when the assay sample contained 500 μg of protein (Fig. 5B). Dexamethasone reversed the effect of cyclophosphamide on [^{3}H]des-Arg^{10}-kallidin binding because, as in control animals, no specific binding was present in cyclophosphamide-treated rats pretreated with dexamethasone (Fig. 5D). With 500 μg of protein in each sample the specific binding was 9 ± 5, 23 ± 2, 9 ± 2 and 8 ± 2% of the total for saline–control, saline–cyclophosphamide, dexamethasone–control and dexamethasone–cyclophosphamide groups, respectively.

Statistically significant specific binding of [^{3}H]-bradykinin (a radioligand for bradykinin B_2 receptors) to urinary bladder membranes was present in all experimental groups when 100 μg of protein was used in the assay sample (Fig. 6A to D). With 200 μg of protein in each sample the specific binding was 68 ± 2, 69 ± 1, 61 ± 1 and 59 ± 2% of the total for saline–control, saline–cyclophosphamide, dexamethasone–control and dexamethasone–cyclophosphamide groups, respectively.

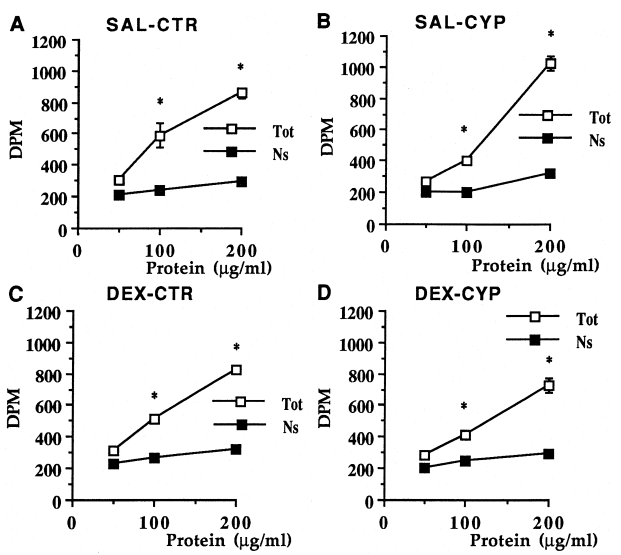

Fig. 6. Effect of saline (SAL, panels A and B) or dexamethasone (DEX, panels C and D) pretreatment in control (CTR, panels A and C) or cyclophosphamide-treated rats (CYP, panels B and D) on [^{3}H]bradykinin binding to urinary bladder membranes as a function of protein content (μg). DPM, disintegrations per min; Tot, total binding (0.5 nM of [^{3}H]bradykinin); Ns, non-specific binding (0.5 nM of [^{3}H]bradykinin and 1 μM of bradykinin. Each point and bar represents the mean ± S.E.M. of four determinations. Fisher's LSD test: $^*P < 0.01$ vs. Ns.

4. Discussion

The present results provide evidence that pretreatment with steroidal anti-inflammatory drugs, like dexamethasone, can afford a certain level of protection against urinary bladder damage in a rat model of cystitis induced by cyclophosphamide. Cyclophosphamide is an alkylating agent which, at high doses, induces haemorrhagic cystitis in humans (Watson and Notley, 1973). Besides bladder inflammation, haemorrhage and oedema, the rat model of cyclophosphamide-induced cystitis is characterized by detrusor hyperreflexia (Maggi et al., 1992; Lecci and Maggi, 1996). In this study we also analyzed the changes in the visco-elastic properties of the bladder wall (decreased compliance) unrelated to nervous reflex activity, which probably represents the animal counterpart of the fibrotic bladder wall described in humans (Watson and Notley, 1973). Dexamethasone completely antagonized the increase in bladder weight induced by cyclophosphamide and this may be partly due to the anti-oedema properties of steroids (Bacci et al., 1993). However, since the cyclophosphamide-induced increase in bladder weight can only partly be attributed to oedema (Maggi et al., 1992), it is also possible that chronic dexamethasone treatment improved bladder compliance in animals with bladder inflammation by preventing the tissue hypertrophy induced by cyclophosphamide. It is, however, noteworthy that in cyclophosphamide-pretreated rats which were chronically treated with dexamethasone, bladder compliance was still lower than in the respective control group, indicating that oedema and the increase in bladder weight are not the only factors affecting bladder compliance.

There is evidence that kinins are involved in bladder inflammation and that the local contractile activity mediated by bradykinin B_1 receptors greatly increases during cystitis. For this reasons we studied whether the decrease in bladder compliance produced by cyclophosphamide involves local kinin formation. Indeed, we found that sustained tonic-type local contractile activity could be elicited by bradykinin B_1 receptor agonists during cyclophosphamide-induced bladder inflammation and that chronic dexamethasone treatment prevented the development of this response, by preventing the inflammation-induced expression of bradykinin B_1 receptors, as indicated by the absence of specific [^{3}H]des-Arg^{10}-kallidin binding in urinary bladder membranes from cyclophosphamide-treated animals pretreated with dexamethasone. As negative control, dexamethasone pretreatment neither reduced the bladder contractions induced by tachykinin NK_2 receptor stimulation, nor inhibited the binding of the bradykinin B_2 receptor radioligand [^{3}H]bradykinin to urinary bladder membranes in any experimental group. Moreover, the acute administration of dexamethasone did not reduce per se the contractile effect evoked by des-Arg^9-bradykinin in both control and inflamed bladders, although the steroid antagonized the time-dependent up-regulation of bradykinin B_1

receptor-mediated contractile responses due to the surgical manipulations necessary for intravesical pressure recording, in both normal and cyclophosphamide-treated rats (Lecci et al., 1998). These results support the concept that anti-inflammatory steroids inhibit the expression of bradykinin B_1 receptors and exclude any direct interference of dexamethasone with bradykinin B_1 receptor-activated transduction mechanisms (Deblois et al., 1988). If we consider that part of the anti-inflammatory effect of steroids is mediated by the synthesis or the release of lipocortin, a protein that inhibits phospholipase A_2 activity (Flower, 1988), and that, after cyclophosphamide pretreatment, the bradykinin B_1 receptor-elicited detrusor contraction does not depend on arachidonic acid metabolites (Meini et al., 1998), the resistance of this response to acute dexamethasone treatment in the bladder inflammation group is not a surprising result. However, in the control group, the time-dependent bradykinin B_1 receptor-mediated bladder contractile response was abolished by cyclooxygenase inhibitors (Meini et al., 1998), which suggests that dexamethasone could have an inhibitory effect. The lack of an inhibitory effect of dexamethasone on bradykinin B_1 receptor-elicited contraction in control animals can be explained either by the presence of dexamethasone-resistant, interleukin-1-stimulated phospholipase A_2 activity (Kol et al., 1998) and/or by the triggering of phosphorylation processes that greatly reduce the inhibitory activity of lipocortin on phospholipase A_2 (Flower, 1988). It is also worth noting that, although a certain level of constitutive expression of bradykinin B_1 receptor mRNA has been detected in the rat urinary bladder (Luccarini et al., 1998; Belichard et al., 1998), no bradykinin B_1 receptor-elicited contractions are evident until 30 min after the surgical procedures necessary for bladder pressure recordings in vivo (Lecci et al., 1998). Furthermore, no specific binding of [^{3}H]des-Arg^{10}-kallidin was detected in urinary bladder membranes from control rats, whereas this binding was present in cyclophosphamide-treated animals. In agreement with the latter finding, it was found that cyclophosphamide-induced bladder inflammation resulted in an increased expression of mRNA for bradykinin B_1 receptors (Luccarini et al., 1998).

Despite the evidence for the expression of bradykinin B_1 receptors mediating bladder smooth muscle contraction after cyclophosphamide-induced inflammation, the selective bradykinin B_1 receptor antagonist des-Arg^{10}-Hoe 140 (Meini et al., 1996) either alone, or in combination with the selective bradykinin B_2 receptor antagonist Hoe 140 (Maggi et al., 1993), did not modify bladder compliance in cyclophosphamide-treated rats. These results exclude that endogenous kinins participate in the maintenance of vesical tone during bladder inflammation, although they do not exclude that the target(s) or the role of kinins may be different from those considered here. For instance, Hoe 140 reduces bladder hyperreflexia and oedema in the cyclophosphamide model (Maggi et al., 1993; Ahluwalia et al., 1994), possibly by blocking the effects of bradykinin acting at bradykinin B_2 receptors located on sensory nerves (Maggi et al., 1993; Ahluwalia et al., 1994; Lecci et al., 1995). Furthermore, in agreement with the above hypothesis, recent evidence implicates bradykinin B_1 and B_2 receptors in the bladder hyperreflexia induced by the intravesical administration of turpentine oil (Jaggar et al., 1998), although the contribution of bradykinin B_1 receptors becomes relevant only some hours after the induction of inflammation.

Given the interactions between dexamethasone pretreatment and the effects of cyclophosphamide, changes in the therapeutic efficacy of this latter drug must be carefully evaluated. For instance, it has been reported that arachidonic acid-elicited apoptosis is reversed by dexamethasone in a model of hepatoma (Iidia et al., 1998), and we know that prostanoids are important mediators of cyclophosphamide-induced inflammation (Meini et al., 1998). However, the combination of cyclophosphamide and steroids is frequently used with the aim to reduce nausea and vomiting during chemotherapy (Stewart, 1996), to improve antiblastic activity in certain solid tumors (Haynes, 1990; Taylor et al., 1997), or to increase the immunosuppressant effect of the single drug in tumors or other diseases of the immune system (Haynes, 1990; Huicochea Grobet et al., 1996). Although the occurrence of haemorrhagic cystitis has been reported following this combined treatment (Huicochea Grobet et al., 1996), no systematic studies are presently available that show whether the protection provided by dexamethasone against cyclophosphamide-induced damage of the urinary bladder is confined to animals or whether it also occurs in human patients.

In conclusion, the present study provides evidence for a preventive action of dexamethasone against the decrease in bladder compliance and the up-regulation of bradykinin B_1 receptor expression following cyclophosphamide treatment. However, the reduced bladder compliance observed in animals with bladder inflammation does not depend on detrusor muscle contraction evoked by the stimulation of bradykinin B_1 and/or B_2 receptors by endogenous kinins.

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