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Effect of glucocorticoids, monokines and growth factors on the spontaneously developing responses of the rabbit isolated aorta to des-Arg^9-bradykinin

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1 The mechanisms modulating the spontaneous induction of contractile responses to agonists of the B_1-receptors for kinins have been studied by submitting the rabbit isolated aorta preparation to various *in vitro* treatments. Des-Arg^9-bradykinin (Des-Arg^9-BK), applied after 6 h of *in vitro* incubation was the standard stimulus to monitor this up-regulation process.

2 Specific inhibition of the development of the contractile response to des-Arg^9-BK was obtained by exposing tissues continuously to dexamethasone, dexamethasone sodium phosphate (DSP) or cortisol, but not to oestradiol. The maximal extent of the inhibition obtained at high concentrations of glucocorticoids was 86%.

3 No gross inhibition of protein synthesis was observed in the presence of DSP as monitored by [^{35}S]-methionine incorporation into incubated pieces of rabbit aorta.

4 *In vivo* pretreatment of rabbits with DSP did not reduce further the development of the responses *in vitro*. DSP applied 15 min before the 6 h recording did not antagonize the contractile effect of the BK fragment.

5 Interleukin 1 (IL-1) and interleukin 2 (IL-2) applied *in vitro* for the first 3 h of incubation increased the development of the contractile response to des-Arg^9-BK.

6 Arachidonic acid (AA), nordihydroguaiaretic acid, tumour necrosis factor-α (TNF) and transforming growth factor-β (TGF-β) failed to influence the spontaneous development of the response to kinins.

7 Continuous exposure to DSP (100 μM) markedly inhibited the action of stimulants in this system: IL-1, IL-2, epidermal growth factor and muramyl dipeptide. Moreover, the presence of AA (30 μM) did not prevent the inhibitory effect of DSP (100 μM).

8 None of the treatments applied singly or in combination modified the contractile response of the rabbit aorta to noradrenaline.

9 The results are discussed in terms of the possible involvement of immunocompetent cells in the up-regulation of vascular responsiveness to B_1 receptor agonists.

Introduction

The rabbit isolated aorta preparation incubated in Krebs solution develops a progressive contractile responsiveness to des-Arg^9-bradykinin (BK), an agonist of the B_1-receptor for kinins, to which it is initially insensitive (Bouthillier *et al.*, 1987). This up-regulation process is dependent upon protein synthesis and is enhanced by several substances applied *in vitro* on intact tissues: bacterial lipopolysaccharide (LPS), muramyl-dipeptide (MDP), phorbol myristate acetate (PMA), epidermal growth factor (EGF) and endothelial cell growth factor (ECGF) (Bouthillier *et al.*, 1987). Treatment with EGF, the most potent stimulus identified, increases both the maximal effect and the apparent affinity of kinins, shifting the concentration-effect curves to the left.

Moreover, *in vivo* significant cardiovascular responses to exogenous des-Arg^9-BK, in the form of transient hypotension, occur only in rabbits pretreated with LPS, MDP or PMA, in decreasing order of efficacy (Regoli *et al.*, 1981; Marceau *et al.*, 1984; Bouthillier *et al.*, 1987). Nitrogen mustard-induced neutropaenia did not prevent the development of

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responses to des-Arg^9-BK in LPS-treated rabbits (Bouthillier *et al.*, 1987).

LPS, MDP and, to a lesser extent, PMA share the common biological property of stimulating the synthesis of certain proteins in macrophages such as surface antigen Mo3e (Todd & Liu, 1986). Moreover it is well known that interleukin-1 (IL-1) and tumour necrosis factor (TNF) production is stimulated by LPS or MDP from macrophage-like cells (Kawakami *et al.*, 1982; Dinarello & Krueger, 1986; Warren *et al.*, 1986). Taken together, this suggests a possible role of tissue macrophages in the development of vascular responses mediated by the B_1-kinin receptors.

The present experiments have been undertaken to characterize further the mechanism of induction of vascular responses to des-Arg^9-BK with respect to the effect of monokines, growth factors and steroid sensitivity. Additional evidence is presented here that factors derived from immunologically competent cells influence positively the up-regulation of tissue response to a typical agonist of the B_1-receptors for kinins.

Methods

Isolated blood vessels

Rabbit aortic strips were prepared and suspended in 5 ml tissues baths containing Krebs solution and their responses to agents were recorded as described (Bouthillier *et al.*, 1987). The longest period between washings with fresh Krebs solution was 90 min.

Protocols

A near maximal concentration of des-Arg^9-BK (1.7 μM, which is approximately the ED_{95} according to Regoli & Barabé, 1980) was used to stimulate the strips at various times of incubation *in vitro*. The progressive nature of the contractile response to des-Arg^9-BK has been verified in all protocols by applying the peptide on each strip after 1 h, 3 h and 6 h of incubation as in Bouthillier *et al.* (1987); however, only the responses recorded at 6 h are presented here as being representative of the process. Tissues were allowed to contract for ten minutes before the washout of stimulant. Contractile responses to noradrenaline (NA, 100 nM) were also monitored at 1.5 h and 6.5 h of incubation. Contractions are expressed in g.

The concentration-effect relationship of steroids on the system was established for dexamethasone, DSP, cortisol and oestradiol. Steroids were present in the bathing fluid throughout the incubation period. In some experiments, tissues were exposed to DSP during specific portions of the *in vitro* incubation. In other experiments, rabbits were pretreated with DSP (6 mg i.v.) 5 h before death; this dose given fractionally to rabbits over a week has been shown to produce a plasma concentration of dexamethasone averaging 100 nM, which results in a >90% occupancy of the glucocorticoid receptors (Náray-Fejes-Tóth *et al.*, 1984). The aortic tissue of DSP-pretreated animals was subsequently incubated *in vitro* for 6.5 h with or without DSP (100 μM).

In order to assess the mechanism of the development of the response to des-Arg^9-BK, several other substances were introduced in tissue baths: NDGA, AA (throughout the incubation period), TNF, IL-1, TGF-β or IL-2 (for the first 3 h). The effect of the kinin fragment was tested at 1, 3 and 6 h in all preparations.

Enhanced up-regulated responses to des-Arg^9-BK were observed in strips of rabbit aorta incubated in the presence of several substances (Bouthillier *et al.*, 1987; present results). In order to test whether DSP retained its inhibitory effect in the presence of those stimulating agents, the following protocol was adopted. Four paired strips were prepared from the same aorta. One tissue was exposed to a combination of DSP and a stimulating agent. Two other tissues were exposed to the separate substances; the last strip served as a control to demonstrate up-regulation of des-Arg^9-BK responsiveness.

[^{35}S]-methionine incorporation

The inhibitory effect of glucocorticoids on the development of responses to kinins was analysed in terms of a possible global inhibition of protein synthesis using [^{35}S]-methionine incorporation in whole tissues. Fragments of fresh aortae (average wet weight: 3 mg) were incubated for 6 h at 37°C (95% O_2 and 5% CO_2) in 3 ml of Krebs containing 10 μCi ml^{-1} of [^{35}S]-methionine (Amersham Corp., Arlington Heights IL; specific activity 800 Ci mol^{-1}) and DSP, oestradiol or cycloheximide. Incubated fragments were rinsed in phosphate buffered saline, pH 7.4, and solubilized overnight at 50°C using the tissue solubilizer Scintigest (90% v/v; Fisher, Fair Lawn, NJ). Aliquots were added to scintillation fluid and radioactivity was determined. Results are expressed as c.p.m. of [^{35}S]-methionine incorporated per mg of wet tissue.

Drugs

The following drugs and compounds were purchased from Sigma (St. Louis, MO): (−)-noradrenaline

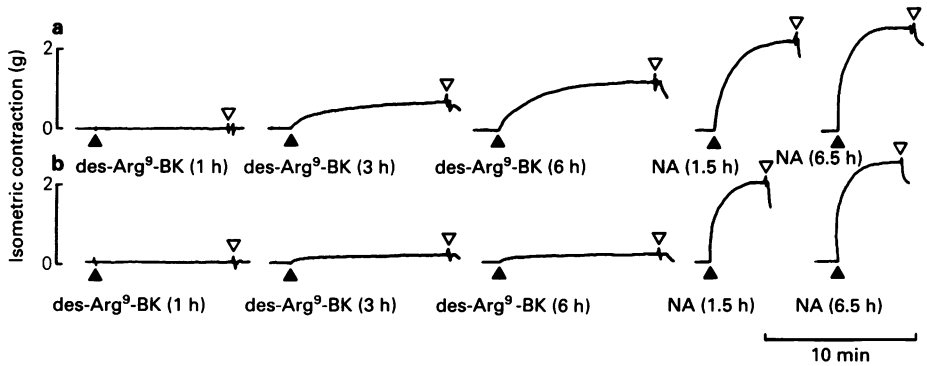

Figure 1 Contractile effect of des-Arg^9-bradykinin (1.7 μM) applied at 1, 3 and 6 h, and of noradrenaline (NA, 100 nM) applied at 1.5 and 6.5 h on rabbit aortic strips. Two strips of the same aorta isolated from a normal rabbit were used. In (b), dexamethasone sodium phosphate (DSP, 100 μM) was present continuously in the bathing solution and inhibited selectively the development of the response to the kinin. (▲) Refers to the application of agents and (▽) to washout of stimulants. Abbreviations used: des-Arg^9-BK, des-Arg^9-bradykinin; NA, noradrenaline.

(NA), dexamethasone, cortisol, oestradiol, cycloheximide, arachidonic acid (AA, from porcine liver, h.p.l.c. repurified), nordihydroguaiaretic acid (NDGA), epidermal growth factor (EGF, from mouse submaxillary glands, receptor grade) and bovine serum albumin. Des-Arg^9-BK was purchased from Bachem (Torrance, CA); dexamethasone sodium phosphate (DSP) was from Sabex (Montréal, Canada); transforming growth factor-β (TGF-β, from porcine platelets) was from R & D Systems (Minneapolis, MN); human tumour necrosis factor-α (TNF, recombinant from *E. coli*), from Amgen Biologicals (Thousand Oaks, CA); interleukin-1β (IL-1, recombinant from *E. coli*), from Cistron Technology (Pine Brooks, NJ). Interleukin-2 (IL-2, T-cell growth factor, from rat splenocytes) was purchased from Collaborative Research (Bedford, MA). One unit of TNF is the amount required to yield 50% lysis of L-929 cells in 48 h in the absence of actinomycin D. One unit of IL-1 is the amount which causes half-maximal incorporation of [^{3}H]-thymidine by murine (C3H) thymocytes in the presence of concanavalin A (0.5 μg ml^{-1}). One unit of IL-2 supports the exponential growth of IL-2-dependent murine T-cells from 0.5×10^4 to 6.5×10^5 cells ml^{-1} over approximately 3 days.

Drugs were dissolved in pyrogen-free 0.9% saline except for dexamethasone, cortisol, oestradiol which were dissolved in 70% ethanol, and AA and NDGA which were dissolved in 90% ethanol. Tissues treated with drugs dissolved in ethanol were paired with controls exposed to an equivalent concentration of the ethanol vehicle (maximum 0.3%). TGF-β was dissolved in saline containing 1 mg ml^{-1} BSA. Drugs were injected in small volumes (5–170 μl) into the tissue baths at the appropriate time and concentrations are expressed at the tissue level.

Statistical analysis

In most protocols, responses obtained in treated tissues were matched with those from paired controls from the same rabbit and results were analysed by means of Student's *t* test for paired samples in order to minimize the effect of inter-animal variation.

Results

Effect of glucocorticoids on the spontaneous sensitization to des-Arg^9-BK

Continuous exposure *in vitro* to dexamethasone, dexamethasone sodium phosphate (DSP) and cortisol inhibited the development of the contractile response to des-Arg^9-BK (1.7 μM) in rabbit aortic strips incubated for 6 h (typical tracings, Figure 1; concentration-effect relationship, Figure 2). Oestradiol was ineffective in this system. Control values for the time-dependent up-regulated contractions to des-Arg^9-BK were established for each steroid using tissues derived from the same aortae used within the group exposed to the glucocorticoid. The inhibition was dose-dependent over the concentration range tested for DSP and cortisol. Dexamethasone was the most potent inhibitor, producing maximum inhibition with the lowest dose tested (100 pM) so a dose-effect relationship was not established. A certain component of the response recorded at 6 h appeared to be steroid-resistant: 86% was the highest level of inhibition obtained even with high doses of glucocorticoids. In contrast exposure of the tissues to steroids, even at the highest concentration tested, had no significant effect on the contractile response

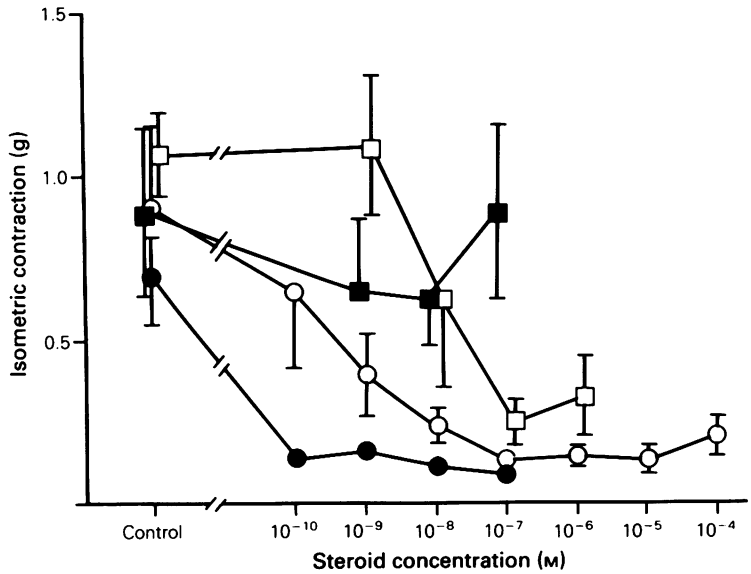

Figure 2 Concentration-effect relationship for the inhibitory effect of steroids on the contractile response to des-Arg^9-bradykinin (1.7 μM) of rabbit aortic strips after 6 h of incubation. Effects of dexamethasone sodium phosphate (○), dexamethasone (●), cortisol (□) and oestradiol (■) are shown. Steroids were present in the bathing fluid throughout the incubation period. Results are the mean of 4 to 9 determinations and are expressed in g of contraction, vertical lines indicate s.e.

to noradrenaline as compared to paired controls (Table 1).

In a different set of experiments, four paired strips were prepared from each aorta and exposed to DSP (100 μM) for different periods of time (Figure 3a). Contractile responses to des-Arg^9-BK were recorded at 6 h in tissues exposed to DSP from the first hour of incubation onwards; the responses were significantly less than in non-steroid exposed controls but significantly higher than those of tissues exposed to the steroid throughout the 6 h incubation period. Late application of DSP (15 min before the 6 h recording) had no effect on the level of contraction to des-Arg^9-BK as compared to controls, suggesting that DSP does not antagonize acutely the effect of kinins. In another set of experiments, 45% inhibition of the contractile effect of des-Arg^9-BK was obtained by exposing tissues to a low concentration of DSP (10 nM; $n = 4$) for the first hour of incubation only; this was less extensive than the 74% inhibition obtained following continuous exposure to the same concentration of DSP (Figure 2). Exposing the tissues to DSP (10 nM or 100 μM) for specific portions of the *in vitro* incubation period did not affect the contractile responses to NA (not shown).

Aortae of rabbits pretreated with DSP (6 mg i.v. 5 h before death) were incubated *in vitro* with or without DSP (100 μM). The maximal responses to des-Arg^9-BK after 6 h in the absence of DSP *in vitro* were significantly lower than those recorded in control strips from untreated rabbits (Figure 3b). When DSP was administered both *in vivo* and *in vitro*, the response to the BK fragment was not significantly different from the one recorded in paired tissues exposed to DSP *in vivo* but not *in vitro*. This suggests that glucocorticoids, unlike cycloheximide, cannot abolish totally this response. The *in vivo* DSP pretreatment did not affect contractions to NA (Table 1).

[^{35}S]-methionine incorporation into incubated rabbit aortic fragments was not affected by continuous exposure to DSP (100 pM–1 μM) or to oestradiol (1–100 nM) but was significantly inhibited by cycloheximide (71 μM; Table 2).

In order to assess any possible contribution of products of the arachidonic acid cascade, the formation of which may be inhibited by glucocorticoids (Blackwell *et al.*, 1980), the dual cyclo-oxygenase and lipoxygenase inhibitor NDGA (100 nM and 1 μM) was introduced in tissue baths throughout the incubation period. It did not have any significant effect on the level of contraction to des-Arg^9-BK at 6 h as compared to paired controls (mean $\pm$ s.e.; 0.40 $\pm$ 0.17 g in tissues treated with 1 μM, NDGA and 0.35 $\pm$ 0.13 g in paired controls; $n = 5$). At 10 μM, NDGA depressed both kinin and NA-induced con-

Table 1 Contractile response of rabbit aortic strips incubated *in vitro* for 6.5 h to noradrenaline (100 nM)[a]

Treatment (*concentration*)	*Contractile response* (g) *Control tissues*	*Treated tissues*
Steroids		
Dexamethasone (100 nM)	2.4 ± 0.2 (4)	2.4 ± 0.1 (4)
DSP (100 μM)	2.6 ± 0.4 (5)	2.0 ± 0.3 (5)
Cortisol (1 μM)	2.6 ± 0.4 (4)	2.3 ± 0.3 (4)
Oestradiol (100 nM)	2.5 ± 0.1 (9)	2.3 ± 0.2 (9)
DSP *in vivo*[b] followed by DSP *in vitro* (100 μM)	2.6 ± 0.2 (8)	2.7 ± 0.2 (8)
Bioactive proteins		
TNF (100 u ml^{-1})	2.7 ± 0.1 (6)	2.6 ± 0.1 (6)
IL-1 (20 u ml^{-1})	2.8 ± 0.4 (4)	2.4 ± 0.4 (4)
TGF-β (25 ng nl^{-1})	2.6 ± 0.3 (5)	2.4 ± 0.4 (5)
IL-2 (50 u ml^{-1})	2.1 ± 0.2 (5)	1.7 ± 0.4 (5)

[a] Treated tissues were exposed to steroids throughout the incubation period or to bioactive proteins for the first 3 h. Student's *t* test for paired data showed no statistical difference between control and treated groups of tissues.
[b] Rabbits were injected with 6 mg of DSP intravenously 5 h before death.
Abbreviations used: DSP, dexamethasone sodium phosphate; TNF, human tumour necrosis factor-α; IL-1, interleukin-1β; IL-2, interleukin 2; TGF-β, transforming growth factor-β.

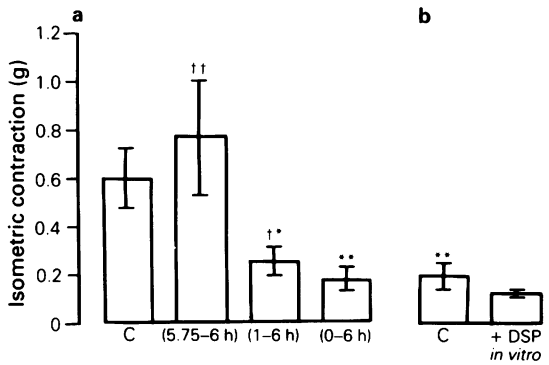

Figure 3 Contractile response to des-Arg^9-bradykinin (1.7 μM) of rabbit aortic strips incubated for 6 h and subjected to various treatments involving dexamethasone sodium phosphate (DSP). (a) Strips were exposed to DSP (100 μM) during specific portions of the *in vitro* incubation: last 15 min (5.75–6 h), last 5 h (1–6 h) or throughout the incubation period (0–6 h). $n = 8$. Analysis of variance followed by Student's *t* test for paired samples showed that the longer exposures to DSP caused a significant inhibition as compared to controls (C; $^*P < 0.05$, $^{**}P < 0.01$). In addition, strips continuously exposed to DSP *in vitro* (0–6 h) responded significantly less than tissues from the two other DSP-treated groups ($\dagger P < 0.05$, $\dagger\dagger P < 0.01$). (b) Aortic strips removed from rabbits pretreated with DSP *in vivo* (6 mg i.v. 5 h before death) were incubated 6 h *in vitro* with or without DSP (100 μM). $n = 8$. Student's *t* test for paired samples showed no difference in the magnitude of contractions between the two groups. Additionally, the responses recorded in the absence of DSP were significantly inferior to the ones recorded with strips from rabbits not treated with DSP (comparison made with the control group in (a), $^{**}P < 0.01$).

tractions suggesting a toxic action on the preparation. Arachidonic acid (AA, 300 nM, 3 μM and 30 μM) also failed to influence the spontaneous increase in response to the BK fragment (0.89 ± 0.18 g in tissues treated with 30 μM AA and 0.78 ± 0.17 g in paired controls, $n = 13$). Moreover, incubation in the presence of AA (30 μM) did not prevent the inhibitory effect of DSP (100 μM, not shown).

Effect of monokines and growth factors

Rabbit aortic strips were incubated for the first 3 h in the presence of recombinant TNF (20 and

Table 2 [^{35}S]-methionine incorporation into pieces of rabbit aorta incubated for 6 h *in vitro*

Treatment		[^{35}S]-*methionine incorporated*[a] (c.p.m. × 10^{-3})
Controls		323 ± 45
Cycloheximide	71 μM	51 ± 8[b]
DSP	1 μM	268 ± 33
	100 nM	281 ± 29
	10 nM	364 ± 63
	1 nM	286 ± 38
	100 pM	298 ± 115
Oestradiol	100 nM	317 ± 33
	10 nM	322 ± 24
	1 nM	387 ± 86

[a] Results are expressed as c.p.m. of ^{35}S incorporated per mg of wet tissue and represent the mean ± s.e. of 5 to 6 determinations. Groups include tissues from at least 4 different animals.
[b] Value significantly different from controls ($P < 0.001$ as calculated by Student's *t* test).

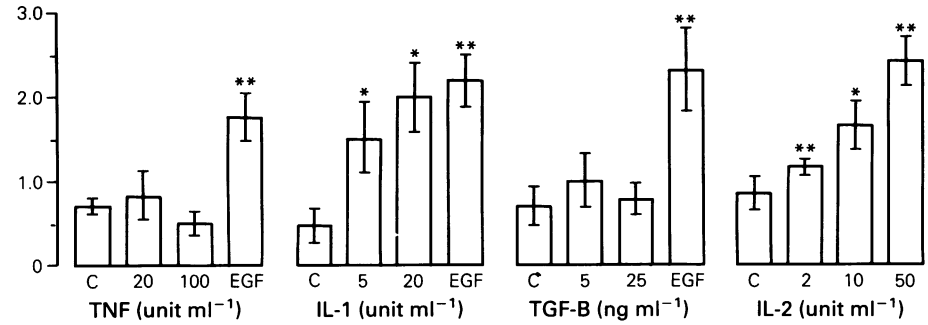

Figure 4 Contractile responses to des-Arg^9-bradykinin (1.7 μM) recorded after 6 h of incubation in groups of aortic strips exposed to biologically active proteins for the first 3 h. In some experiments, positive controls of stimulation were obtained by exposing tissues to epidermal growth factor (EGF, 100 ng ml^{-1}). Results are the mean of 4 to 6 determinations and are expressed in g of contraction; vertical lines indicate s.e. Student's t test for paired samples indicated values different from controls (C) following several treatments (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Abbreviations used: TNF, tumour necrosis factor; IL-1, interleukin-1; TGF-β, transforming growth factor-β; IL-2, interleukin-2.

100 u ml^{-1}), recombinant IL-1 (5 and 20 u ml^{-1}), purified TGF-β (5 and 25 ng ml^{-1}) or purified IL-2 (2, 10 and 50 u ml^{-1}) in order to investigate the action of these biologically active proteins on the developing response to kinins. The concentrations used were chosen as they have been found to influence various systems involving cell culture. Levels of contraction to des-Arg^9-BK recorded at 6 h on strips exposed to IL-1 and IL-2 were significantly higher than paired controls at all concentrations tested (Figure 4). By contrast, TNF and TGF-β did not modify the magnitude of the response to the octapeptide. Positive controls were obtained in the groups of tissues exposed to TNF, IL-1 and TGF-β by exposing paired tissues to EGF (100 ng ml^{-1}, first 3 h of incubation), a potent stimulant in this system (Bouthillier *et al.*, 1987). Contractions to NA were not affected by the treatments with monokines and growth factors (Table 1).

We have tested the sensitivity to glucocorticoids of vascular responses to des-Arg^9-BK under stimulation with the active cytokines IL-1 and IL-2, or EGF or the macrophage activator MDP; the latter substance has also been shown to increase the effect of kinins on the rabbit aorta preparation (Bouthillier *et al.*, 1987). DSP (100 μM) inhibited significantly the stimulating effect of IL-1 (10 u ml^{-1}), IL-2 (10 u ml^{-1}), MDP (2 μg ml^{-1}) and EGF (100 ng ml^{-1}) (Figure 5). However, the inhibition by DSP was not as great when combined with IL-1 as when combined with the other stimulants: the responses to des-Arg^9-BK of tissues treated with IL-1 and DSP together were significantly greater than the ones of tissues exposed to DSP alone ($P < 0.05$).

Concentration-effect curves of des-Arg^9-BK in the presence of DSP, IL-1 or IL-2

Complete concentration-effect curves for des-Arg^9-BK were established using a cumulative protocol (Regoli *et al.*, 1978) after 6 h of incubation in the presence of DSP, IL-1 or IL-2 (Figure 6). This analysis appeared useful because the response to a single concentration of the peptide might not reflect possible changes in sensitivity of the preparation (expressed as ED_{50}, for instance). As already found using single doses of des-Arg^9-BK, the curves showed that the maximal responses to the peptide were positively (IL-1, IL-2) or negatively (DSP) influenced by the treatments (Figure 6a). However, the concentration-effect curves expressed as percentage of the maximal response seemed to be shifted to the left in proportion to the increase of maximal response for the two types of interleukins (Figure 6b). A similar observation has been described for another strong stimulant: EGF at 100 ng ml^{-1} (Bouthillier *et al.*, 1987). DSP had a selective effect on the maximal response and did not affect the apparent affinity of the kinin for B_1-receptors (Figure 6b).

Discussion

In the present study, several treatments were found to modulate positively or negatively the spontaneous increase in responsiveness to des-Arg^9-BK that occurs when rabbit aortic strips are incubated for several hours *in vitro*.

Our results demonstrate that glucocorticoids specifically inhibit the development of responses to des-Arg^9-BK of the incubated rabbit aorta. The relative

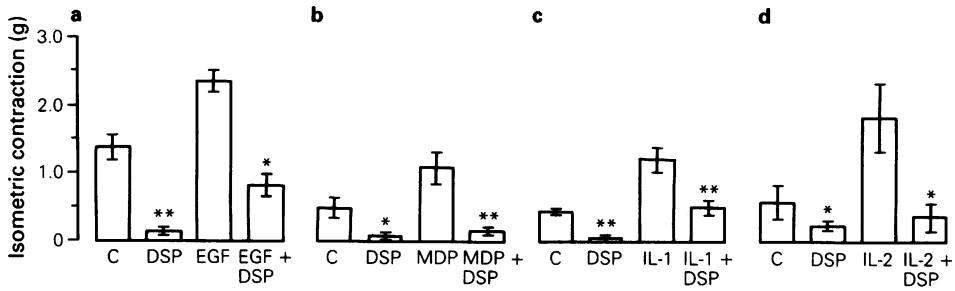

Figure 5 Contractile responses to des-Arg^9-bradykinin (1.7 μM) of rabbit aortic strips incubated for 6 h and exposed to dexamethasone sodium phosphate (DSP, 100 μM) throughout the incubation period or to a stimulating factor during the first 3 h only (EGF, 100 ng ml^{-1}; MDP, 2 μg ml^{-1}; IL-1, 10 u ml^{-1}; IL-2, 10 u ml^{-1}) or to a combination of the two types of treatment. In each group of tissues, one preparation serves as a control (C) for the spontaneous up-regulation of response. Results are the means of 4 to 9 determinations and are expressed in g of contraction; vertical lines indicate s.e. Values of spontaneous and stimulated up-regulations were compared with the corresponding groups exposed to DSP by use of Student's t test for paired samples and the statistical differences are expressed as * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. Abbreviations used: EGF, epidermal growth factor; MDP, muramyl dipeptide; IL-1, interleukin-1; IL-2, interleukin-2.

order of inhibitory potency of the steroids tested is consistent with their glucocorticoid activity, dexamethasone being more potent than cortisol (Haynes & Murad, 1985). DSP is a hydrosoluble ester of dexamethasone that is likely to cross membranes less effectively than the original steroid; we found that DSP was somewhat less potent than dexamethasone in preventing the development of the response to des-Arg^9-BK but it was preferred to dexamethasone in subsequent experiments because no alcohol was needed to dissolve it. Application of DSP for only 15 min before the 6 h test with des-Arg^9-BK did not affect the kinin contractions, indicating that the glucocorticoid does not antagonize acutely the effect of the BK fragment. In fact, it is well known that most, if not all, actions of glucocorticoids are mediated through changes in gene transcription and protein synthesis (Harrison, 1983; Rousseau, 1984; Lan *et al.*, 1984) and a 15 min contact period is probably too short to initiate such a change.

Protein synthesis is necessary for the up-regulation of contractile or relaxant responses to des-Arg^9-BK in rabbit isolated blood vessels (Regoli *et al.*, 1978; Whalley *et al.*, 1983; Bouthillier *et al.*, 1987; Deblois & Marceau, 1987). DSP did not inhibit [^{35}S]-methionine incorporation into incubated pieces of rabbit aorta, indicating that the inhibitory effect of glucocorticoids in this system was not associated with gross inhibition of protein synthesis. It is assumed that most of the incorporated labelled amino acid was associated with newly formed proteins and not present in the cytosol in the form of a free monomer; this assumption appears to be valid because cycloheximide inhibited most of the incorporation of label. This approach does not indicate whether the synthesis of specific proteins was affected by DSP.

Complete inhibition of the response to des-Arg^9-BK was not attained even with high concentrations of glucocorticoids (Figures 1 and 2). It is possible that the time lag necessary for steroid action *in vitro* was sufficient to permit the development of a certain level of responsiveness to des-Arg^9-BK in incubated tissues. However, the results obtained with aortae of rabbits pretreated with a large dose of DSP *in vivo* do not support this explanation: aortic strips from those animals contracted in response to des-Arg^9-BK, though with a decreased amplitude, even when DSP was maintained *in vitro* (Figure 3b). Therefore, a component of the response to the octapeptide appears to be resistant to the inhibitory action of steroids. This residual response is not present initially, since there was no response at all to des-Arg^9-BK if the peptide was applied after only 1 h of incubation (Figure 1).

Glucocorticoids, such as cortisol, stimulate the synthesis of a class of proteins showing inhibitory properties for phospholipase A_2 (e.g. macrocortin, Blackwell *et al.*, 1980). Thus the inhibition of the whole cascade of arachidonic acid (AA) metabolism may result from the action of glucocorticoids. Continuous exposure to indomethacin failed to inhibit the development of contractile responses to des-Arg^9-BK in the rabbit isolated mesenteric vein in a previous study (Regoli *et al.*, 1978). In the present experiments, incubating rabbit aortic strips in the presence of AA or of the dual cyclo-oxygenase and lipoxygenase inhibitor NDGA did not have any specific effect on the contractions to des-Arg^9-BK at 6 h. Moreover, incubation of the tissues in the presence

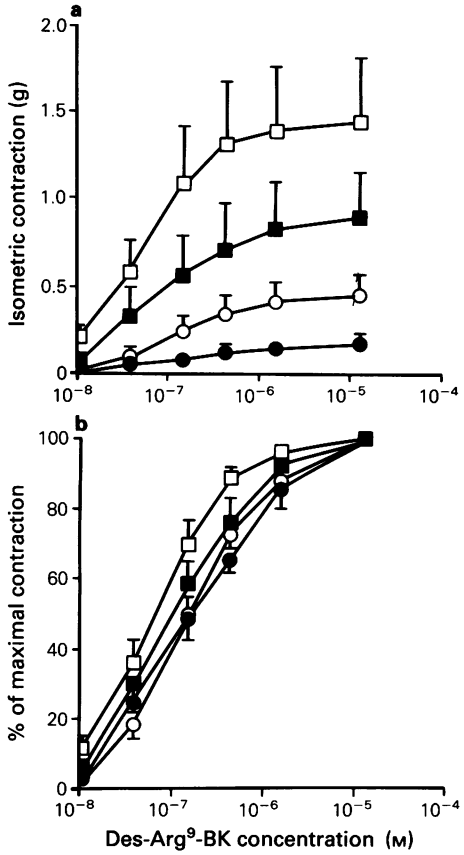

Figure 6 Concentration-effect curves for des-Arg^9-bradykinin on (○) control rabbit aortic strips and on tissues continuously exposed to (●) dexamethasone sodium phosphate (100 μM), (□) interleukin-1 (10 u ml^{-1}), or (■) interleukin-2 (10 u ml^{-1}). The points represent the mean of 5 determinations made after 6 h of incubation, and vertical lines show s.e. mean. The curves presented in (a) are expressed as g of contraction. (b) Represents the same data as a % of the maximal contraction.

of AA did not prevent the inhibitory effect of DSP. Taken together, these results suggest that the up-regulation of the response to the peptide is not associated with AA metabolism.

Interleukin 1 (IL-1), a key mediator in host response to infection and inflammation (Dinarello, 1984), was a very potent stimulator of responses to des-Arg^9-BK in our system. The two most effective substances known to induce a state of responsiveness to des-Arg^9-BK *in vivo* in the rabbit are LPS and MDP (Bouthillier *et al.*, 1987), two pyrogenic substances that promote the synthesis and release of IL-1 from macrophage-like cells (Dinarello & Krueger, 1986; Warren *et al.*, 1986) and guinea-pig fibroblasts (Iribe *et al.*, 1983). IL-1 is also released by LPS treatment of human endothelial cells (Miossec *et al.*, 1986) and human vascular smooth muscle cells (Linny *et al.*, 1986). LPS-stimulated macrophages also release TNF (Kawakami *et al.*, 1982). However, this monokine had no effect on the up-regulation of responses to des-Arg^9-BK of rabbit aortic strips, even at concentrations known to be active in several *in vitro* systems.

The obligatory role of IL-1 in the process of up-regulated responses to des-Arg^9-BK in aortic strips would provide a possible explanation of the inhibitory effect of glucocorticoids on the latter phenomenon: IL-1 synthesis is inhibited by low concentrations of these steroids (Smith, 1980). IL-2, also known as T-cell growth factor, was also identified as a potent stimulant of the up-regulation of responses to des-Arg^9-BK in this system. The presence of T lymphocytes in isolated aortic strips has not been determined but there is a possibility that a certain number might have been sequestrated in the vascular tissue during the isolation procedure. It is well known that IL-1 stimulates IL-2 production by T cells (e.g. Lowenthal *et al.*, 1986), thus suggesting that IL-2 synthesis might be a further necessary step in the up-regulation process. However, it is not clear whether such a chain of mediators is involved. Alternatively, secretions from lymphocytes stimulated with IL-2 might further activate tissue macrophages: colony stimulating factors secreted by lymphocytes have been shown to induce the production of IL-1 by macrophages (Moore *et al.*, 1980). In addition, the stimulatory effect of IL-2 in our system may be due to a direct effect on cell types different from lymphocytes such as macrophages, smooth muscle cells or fibroblasts. In fact, the presence of IL-2 receptors has been reported recently on human monocytes stimulated with LPS (Holter *et al.*, 1987). These cells responded to IL-2 stimulation with an enhanced H_2O_2 production.

TGF-β, a potent regulator of cellular proliferation and differentiation, is synthesized notably by activated lymphocytes and blocks the stimulatory effect of IL-2 in this cell type (Kherl *et al.*, 1986). It had no effect on the spontaneous development of the contractile response to des-Arg^9-BK. This constitutes further evidence against the obligatory role of IL-2 in the process.

The inhibition by DSP of the amplified response to des-Arg^9-BK in the presence of stimulators of IL-1 production, such as MDP, is consistent with an obligatory role for IL-1 in this system. However, it is not easy to explain why the effect of all stimulants tested, including IL-1, are inhibited by DSP, although IL-1 treated tissues might have been more resistant to inhibition. The ultimate tissue requirement for des-Arg^9-BK activity as postulated by

Regoli *et al.* (1978), the *de novo* synthesis of B_1 receptors, is perhaps itself sensitive to the action of glucocorticoids. More work involving pure cell types will be needed to dissect further the mechanism of induction of responses to agonists of the B_1 receptors for kinins.

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