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Short communication

Retinoids inhibit bradykinin B_1 receptor-sensitized responses in human umbilical vein

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Abstract

Bradykinin B_1 receptors are not expressed under physiological conditions but are induced under inflammatory conditions. In isolated human umbilical vein, a spontaneous bradykinin B_1 receptor sensitization process has been demonstrated. On the other hand, retinoids have been shown to exert anti-inflammatory and immunomodulatory actions. We have now examined the effects of all-*trans*-retinoic acid and 9-*cis*-retinoic acid on the bradykinin B_1 receptor-sensitized responses in human umbilical vein. Both retinoids produced a concentration-dependent rightward shift of the concentration–response curves for the bradykinin B_1 receptor agonist, des-Arg^9-bradykinin. Retinoid treatment did not modify the responses to bradykinin B_1 receptor-unrelated agonists, bradykinin or serotonin. In conclusion, retinoids inhibit bradykinin B_1 receptor-sensitized responses and this action could participate in their anti-inflammatory and immunomodulatory effects.

Keywords: Bradykinin B_1 receptor; Umbilical vein, human; Retinoid

1. Introduction

In the isolated human umbilical vein, a spontaneous bradykinin B_1 receptor up-regulation process has been demonstrated after a prolonged in vitro incubation (Sardi et al., 1997). Although the regulation of bradykinin B_1 receptor number is presently unknown, several lines of evidence suggest that they are mainly regulated at the transcriptional level, involving nuclear factor-κB (NF-κB) and activation protein-1 (AP-1) binding sites within its promoter region (Bachvarov et al., 1996; Yang and Polgar, 1996; Ni et al., 1998; Sardi et al., 1999).

Retinoids affect cell growth and differentiation, and exhibit anti-inflammatory and immunomodulatory properties (Orfanos and Bauer, 1983; Fumarulo et al., 1991). These effects are achieved through their ability to regulate complex programs of gene expression in target cells by binding to nuclear receptors (Manglesdorf et al., 1994). Moreover, retinoids have been shown to repress NF-κB and AP-1 pathways (Yang-Yen et al., 1991; Gille et al., 1997; Diaz et al., 2000). In the present study, we have examined the inhibitory effects of two biologically active retinoids, all-*trans*-retinoic acid and 9-*cis*-retinoic acid, on the bradykinin B_1 receptor sensitization process in isolated human umbilical vein.

2. Materials and methods

2.1. Concentration–response curves with human umbilical vein rings

Human umbilical cords obtained from normal full-term deliveries were placed in modified Krebs solution at 4°C (of the following mM composition: NaCl 119, KCl 4.7, $NaHCO_3$ 25, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.0, EDTA 0.004, D-glucose 11). Written informed consent was obtained from each parturient woman. The veins were carefully dissected out of the cords and cut into rings of approximately 3-mm width. The preparations were suspended in 10-ml organ baths and stretched with an initial tension of 3–5 g as described previously (Errasti et al., 1999). The tissues were incubated with captopril (1 μM) 30 min before bradykinin receptor stimulation to avoid

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peptide degradation by kininase II (angiotensin converting enzyme).

After a 5-h incubation period, cumulative concentration–response curves were obtained for des-Arg^9-bradykinin (bradykinin B_1 receptor-selective agonist), bradykinin (bradykinin B_2 receptor-selective agonist) or serotonin. Only one agonist concentration–response curve was obtained with each ring. Some human umbilical vein rings were continuously exposed to all-*trans*-retinoic acid (0.1, 1 μM), or 9-*cis*-retinoic acid (0.1, 1 μM) for the 5-h equilibration period, before cumulative addition of the agonist. Other tissues were incubated in presence of these retinoic acids for the last 30 min before constructing cumulative concentration–response curves to the bradykinin B_1 receptor agonist.

2.2. Chemicals

Captopril and 5-HT creatinine sulfate were from Sigma (St. Louis, MO, USA). Bradykinin and des-Arg^9-bradykinin were from Bachem Bioscience (Torrance, CA, USA). All-*trans*-retinoic acid and 9-*cis*-retinoic acid were from Biomol Research Laboratories (Plymouth Meeting, PA, USA).

2.3. Expression of results and statistical analysis

All data are presented as means ± S.E.M. Responses are expressed as grams of developed contraction. The pEC_{50} values, negative logarithm of the agonist concentration that produced 50% of the maximum, were determined and compared using ALLFIT as described previously (Sardi et al., 1997).

3. Results

3.1. Effects of retinoids on bradykinin B_1 receptor sensitization in human umbilical vein

Human umbilical vein rings were exposed to either all-*trans*-retinoic acid (0.1, 1 μM) or 9-*cis*-retinoic acid (0.1, 1 μM) for 5 h in order to evaluate their possible effects on the bradykinin B_1 receptor-sensitized responses. Both all-*trans*-retinoic acid and 9-*cis*-retinoic acid produced a concentration-dependent rightward shift of the concentration–response curves for des-Arg^9-bradykinin without reduction of the maximal responses (Fig. 1, Table 1).

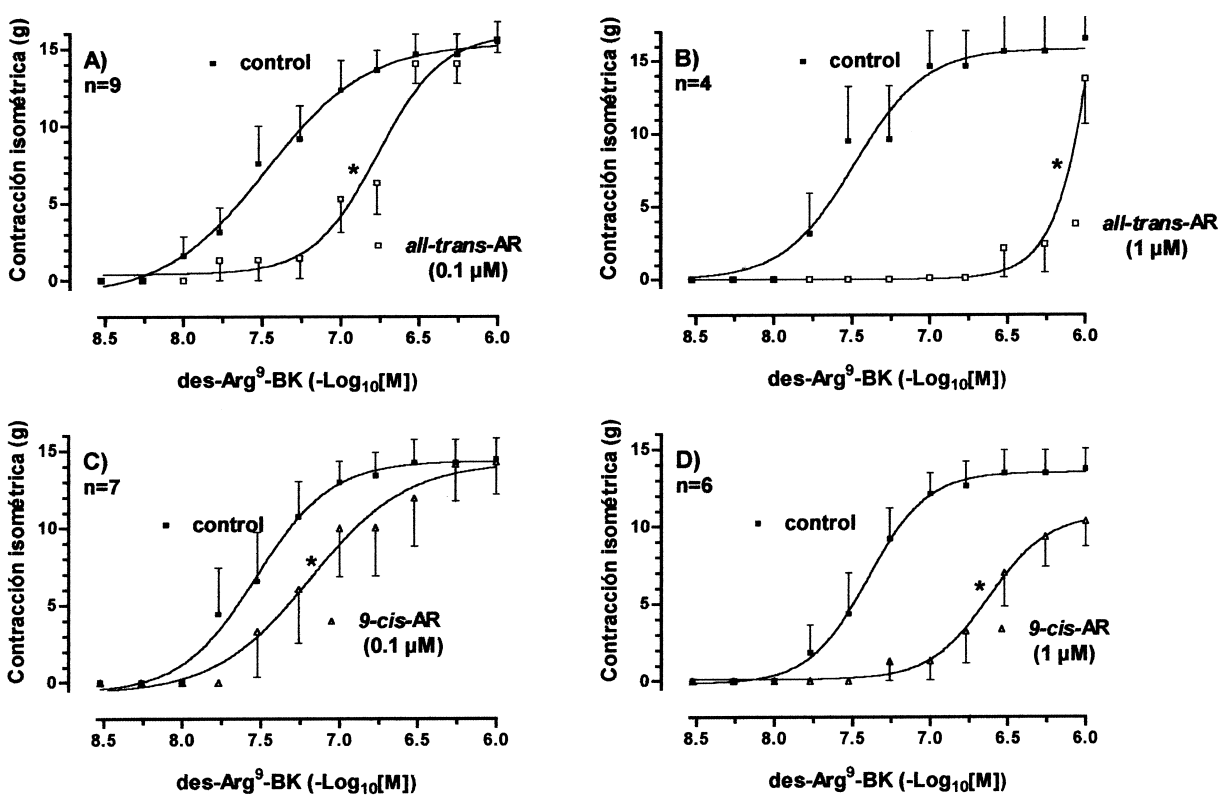

Fig. 1. Concentration–response curves for des-Arg^9-bradykinin on control human umbilical vein rings (■) and on tissues continuously exposed to all-*trans*-retinoic acid (□; A: 0.1 μM, $n = 9$; B: 1 μM, $n = 4$) or 9-*cis*-retinoic acid (△; C: 0.1 μM, $n = 7$; D: 1 μM, $n = 6$). The points represent the means of n determinations made after a 5-h equilibration period and the vertical lines show S.E.M. The responses are expressed in grams of developed contraction. Abscissa scale: $-\log_{10}$ of molar concentration. Symbol (*) represents significant differences ($P < 0.05$) between pEC_{50}.

Table 1
Effects of retinoids (5 h) on the concentration–response curves for des-Arg^9-bradykinin, bradykinin and serotonin in human umbilical vein

Treatment	Agonist	pEC_{50}		Maximal response (g)		*n*
		Control	Treated	Control	Treated	
All-*trans*-retinoic acid (0.1 μM)	des-Arg^9-bradykinin	7.41 ± 0.11	6.77 ± 0.07 *	15.5 ± 1.3	15.6 ± 0.8	9
	bradykinin	9.31 ± 0.10	9.11 ± 0.06	15.5 ± 2.5	17.1 ± 2.2	7
	5-HT	8.27 ± 0.10	8.09 ± 0.06	16.9 ± 2.5	18.0 ± 2.3	7
All-*trans*-retinoic acid (1 μM)	des-Arg^9-bradykinin	7.49 ± 0.10	6.14 ± 0.09 *	16.5 ± 2.7	13.8 ± 3.1	4
	bradykinin	9.30 ± 0.08	9.41 ± 0.10	18.7 ± 3.0	18.2 ± 3.1	5
	5-HT	8.36 ± 0.09	8.06 ± 0.07	13.5 ± 2.6	15.2 ± 1.5	4
9-*cis*-retinoic acid (0.1 μM)	des-Arg^9-bradykinin	7.53 ± 0.10	7.18 ± 0.17 *	14.5 ± 1.4	14.3 ± 2.1	7
	bradykinin	9.59 ± 0.09	9.35 ± 0.09	16.2 ± 2.5	13.9 ± 1.3	4
	5-HT	8.41 ± 0.13	8.69 ± 0.08	18.5 ± 1.3	15.0 ± 2.0	5
9-*cis*-retinoic acid (1 μM)	des-Arg^9-bradykinin	7.40 ± 0.07	6.62 ± 0.10 *	13.7 ± 1.4	10.3 ± 1.6	6
	bradykinin	9.38 ± 0.07	9.59 ± 0.05	16.6 ± 1.3	13.9 ± 0.9	6
	5-HT	8.41 ± 0.13	8.49 ± 0.13	18.5 ± 1.3	14.3 ± 1.4	5

* Represents significant difference from control paired tissues ($P < 0.05$).

To rule out any non-specific or toxic effect of the retinoids, two different treatments were evaluated. Some human umbilical vein rings were incubated with all-*trans*-retinoic acid (1 μM) or 9-*cis*-retinoic acid (1 μM) 30 min before the concentration–response curves to the bradykinin B_1 selective agonist were obtained at 5 h. The concentration–response curves for des-Arg^9-bradykinin were not modified by any of these retinoid treatments (control: 7.47 ± 0.14, all-*trans*-retinoic acid (1 μM): 7.46 ± 0.23, $n = 5$; control: 7.57 ± 0.17, 9-*cis*-retinoic acid (1 μM): 7.31 ± 0.16, $n = 6$). Additionally, after continuous exposure to the retinoic acids (0.1, 1 μM), concentration–response curves to bradykinin B_1 receptor-unrelated agonists such as bradykinin (bradykinin B_2 receptor agonist) or serotonin were made with 5-h incubated tissues. None of the retinoids modified the concentration–response curves for these agonists (Table 1). On the other hand, when applied to tissues incubated for 4.5 h, acute retinoid treatment did not modify the vascular tone.

4. Discussion

The isolated human umbilical vein develops a spontaneous bradykinin B_1 receptor sensitization process. It has been shown that contractile responses to the bradykinin B_1 receptor agonist, des-Arg^9-bradykinin, increase as a function of incubation time (Sardi et al., 1997). Furthermore, in this human tissue, the bradykinin B_1 sensitization phenomenon is inhibited either by actinomycin D, a transcription inhibitor, or by cycloheximide, a protein synthesis inhibitor, and is dependent on trafficking across the Golgi apparatus, as it is blocked by brefeldin A (Sardi et al., 2000).

The mechanism of the rapid bradykinin B_1 receptor induction is still not completely understood. The 5′-flanking region of the human bradykinin B_1 receptor gene bears putative NF-κB as well as numerous AP-1 binding motifs, a promoter organization consistent with activation by cytokines such as interleukin-1β or tumor necrosis factor-α (Bachvarov et al., 1996). We have shown that in the human umbilical vein, bradykinin B_1-mediated responses are potentiated by lipopolysaccharide, interleukin-1β and tumor necrosis factor-α and inhibited by pyrrolidine-dithiocharbamate an antioxidant and NF-κB activation inhibitor (Sardi et al., 1998, 1999).

Retinoids have anti-inflammatory and immunomodulatory actions (Orfanos and Bauer, 1983; Fumarulo et al., 1991). They appear to exert their biological effects through multiple gene regulatory nuclear receptors: the retinoic acid receptors (α, β, and γ) and the retinoid X receptors (α, β, and γ) (Manglesdorf et al., 1994). Alternatively, retinoids can repress the activity of other nuclear transcription factors, such as AP-1 and NF-κB by mechanisms not completely understood (Yang-Yen et al., 1991, Gille et al., 1997, Diaz et al., 2000).

We have examined the action of two biologically active retinoids: all-*trans*-retinoic acid, ligand for retinoic acid receptors, and 9-*cis*-retinoic acid that binds both retinoid receptors (Napoli, 1996). In the human umbilical vein, continuous exposure to all-*trans*-retinoic acid or to 9-*cis*-retinoic acid produced a concentration-dependent inhibition of the bradykinin B_1-sensitized contractile responses, without reduction of the maximal response. The lack of modification of the maximal response to des-Arg^9-bradykinin after the treatment with retinoids suggests the presence of bradykinin B_1 spare receptors in the human umbilical vein after 5 h of incubation, as previously proposed (Sardi et al., 1998, 1999). Then, only if the number of the bradykinin B_1 receptors has been reduced further than the spare receptor population could an appreciable fall in the maximal response be obtained. Furthermore, to rule out any non-specific or toxic effects of the retinoids, different treatments were evaluated. The exposure to all-*trans*-retinoic acid or 9-*cis*-retinoic acid for the last 30 min did not modify the concentration–response curves for des-

Arg^9-bradykinin. In addition, concentration–response curves for agonists unrelated to the bradykinin B_1 receptor were not modified by continuous exposure to retinoic acids. Thus, the retinoid inhibitory effect on bradykinin B_1 sensitized responses appears to be specific.

Bradykinin B_1 receptors are not normally expressed under physiological conditions but are induced in situations of stress, such as septic shock and inflammation (Marceau et al., 1998; Sardi et al., 2000). There is evidence that bradykinin B_1 receptor activation might be involved in mediating the cardinal signs of inflammation: oedema, inflammatory pain, and cellular migration and accumulation (Ahluwalia and Perretti, 1999). To our knowledge, the present study represents the first work showing that retinoids inhibit bradykinin B_1 receptor-sensitized responses. This action might be involved in the anti-inflammatory and immunomodulatory effects of retinoids.

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