Prostaglandins, Leukotrienes and Essential Fatty Acids (2001) **65**(1), 9–14
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doi:10.1054/plef.2001.0280, available online at http://www.idealibrary.com on IDEAL®

Bradykinin and electrical stimulation increase prostaglandin production in the rat vas deferens

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Summary The epididymal portion of the rat vas deferens produced prostaglandins (PG) E_2, $F_{2\alpha}$ and 6-keto $F_{1\alpha}$. Electrical stimulation (ES, 0.1 Hz, 1 ms) increased such production by 100%, and similar results were obtained in the presence of 1.0 μM bradykinin (Bk). When both stimuli were applied simultaneously, the increases in PG production were 1100% for PGE_2, 800% for $PGF_{2\alpha}$ and 400% for PG6-keto $F_{1\alpha}$. Prazosin abolished the effect of ES on PG production. A selective Bk B_2-receptor antagonist abolished the increase in PG production induced by Bk, both in non-stimulated and in ES tissues. Bk (1.0 μM) elicited contractile responses in non-stimulated as well as in ES tissues, responses that were not modified in the presence of 10 μM indomethacin. In conclusion, the effects of Bk on prostaglandin production appears to depend on the activation of B_2 receptors, while the increase in prostaglandin release induced by ES, and the effects observed with both stimuli simultaneously, should be mediated by the release of noradrenaline and the subsequent activation of α_1 adrenoceptors. © 2001 Harcourt Publishers Ltd

INTRODUCTION

Adrenergic stimulation induced either by nerve stimulation[1–3] or by the action of exogenous adrenoceptor agonists[2–6] enhances the synthesis and outflow of prostaglandins in a variety of sympathetically innervated tissues. The released prostaglandins in turn could exert modulatory transynaptic effects on the overflow of the sympathetic neurotransmitter[4,8,9] or produce postsynaptic effects such as facilitation of smooth muscle contractility.[6,10,11]

Bradykinin (Bk), a locally formed nonapeptide with multiple biological effects,[12] enhances the release of noradrenaline in several isolated preparations such as the rat and mouse vas deferens[13] and the rat and human atrium.[14,15] At least in the rat and human atrium, the facilitatory action of Bk on noradrenaline overflow may be mediated by prostaglandins. Moreover, prostaglandins of the E series appear to participate of the inhibitory effect of Bk on noradrenaline release in the rabbit pulmonary artery and heart.[16] The ability of Bk to induce or increase the formation of prostaglandins has been documented in many tissues, namely airways,[17] blood vessels,[18,19] gallbladder[20] and uterus.[21]

Since prostaglandins are involved in the modulation of sympathetic neurotransmission and also may mediate the effects of Bk on the release of sympathetic neurotransmitter, we decided to examine the effect of Bk on prostaglandin production in the electrically stimulated epidydimal portion of the rat vas deferens, an organ with a rich adrenergic innervation[22] and high production of prostaglandins.[23] The type of receptors involved in the effects of Bk and electrical stimulation on prostaglandin efflux were assessed by use of selective antagonists.

Received 2 February 2001
Accepted 18 May 2001

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Grants: This work was supported by Grant No 4041 from CONICET, Argentina.

METHODS

Male Wistar rats of 250–300 g body weight were killed by cervical dislocation. The vasa deferentia were removed and cleaned of connective and fat tissues and the lumen was flushed with 1 ml Krebs solution.

Isolated vas deferens preparation and experimental design

The epididymal portion of each vas deferens was suspended in a 5 ml organ bath containing Krebs solution at 37°C, bubbled with 95% O_2 plus 5% CO_2. The composition of the Krebs solution (mM) was the following: NaCl, 118.0; KCl, 4.7; $CaCl_2$, 2.6; $MgCl_2$, 1.2; NaH_2PO_4, 1.0; $NaHCO_3$, 25.0; glucose, 11.1; EDTA, 0.004; ascorbic acid, 0.11.

The upper end of the vas deferens was connected to a Grass FT-03 force transducer and the isometric tension was recorded on a Grass polygraph model 7-B. The resting tension was adjusted to 1 g and the Krebs solution was renewed every 10 min during 30 min before the start of the experiment.

To determine the effects of 1 μM Bk on the production of prostaglandins, the tissues were incubated in the presence of the drug for periods of 30 min. At the end of each incubation period, the medium was collected and frozen at −20°C for subsequent prostaglandin (PG) extraction. At the end of the experiment, the tissues were blotted dry and weighed.

In another series of experiments, the tissues were field stimulated for 35 min (0.1 Hz, 1 msec, supramaximal voltage) through two parallel platinum electrodes connected to an S44 Grass stimulator. When the electrical stimulation was performed on tissues exposed to 1.0 μM Bk, the drug was added to the organ bath 5 min after the beginning of the stimulation and remained for the next 30 min. At the end of the stimulation, the incubation medium was collected and the tissue was weighed as described above.

Adrenoceptor blocking drugs and Bk-receptor antagonists were added 20 min prior to the start of the electrical stimulation or 25 min prior to the addition of Bk. The prostanoid synthesis inhibitor indomethacin was added 30 min prior to Bk. The drugs remained in the incubation medium until the end of the experiment.

Prostaglandin assay

As previously described,[24] the incubation medium samples were acidified to pH 3.5 with 1.0 M formic acid and extracted 3 times with 2 volumes of chloroform. The chloroform fractions were pooled and evaporated to dryness. Reversed-phase HPLC was carried out on a C18 column (Hibar, E. Merck, 250 × 4 mm, 5 μ). The solvent system was 1.7 mM PO_4H_3 67.2 : acetonitrile 32.8, v/v. The flow rate was 1 ml.min^{-1} and UV absorption was measured at 218 nm. Dried samples were resuspended in 0.15 ml mobile phase and injected into the HPLC system. Authentic standards of PGs: 6-keto PG $F_{1\alpha}$, PGE_2 and $PGF_{2\alpha}$ were run along with the samples, and a bracket assay was performed to determine the amount of PGs in the samples. All values were corrected for recovery loss as determined by parallel standards. Results were expressed as ng of PG per mg of tissue weight. Statistical analysis was performed by ANOVA. At significantly different F values, the differences between the groups were checked by the Tukey test. P levels <0.05 were regarded as significant.

Drugs

Prostaglandins E_2, $F_{2\alpha}$ tris salt and 6-keto $F_{1\alpha}$; bradykinin acetate salt, des-Arg^9, (Leu^8)-bradykinin, D-Arg-(Hyp^3, D-Phe^7)-bradykinin and indomethacin were purchased from Sigma Chemical Co., USA. The following drugs were kindly supplied: (-)-propranolol HCI (ICI Ltd, UK); yohimbine HCI (Schering, Argentina) and prazosin HCl (Pfizer, Argentina). Indomethacin was dissolved in absolute ethanol; the final concentration of ethanol in the incubation medium was 0.1% v/v. Other drugs were dissolved in distilled water.

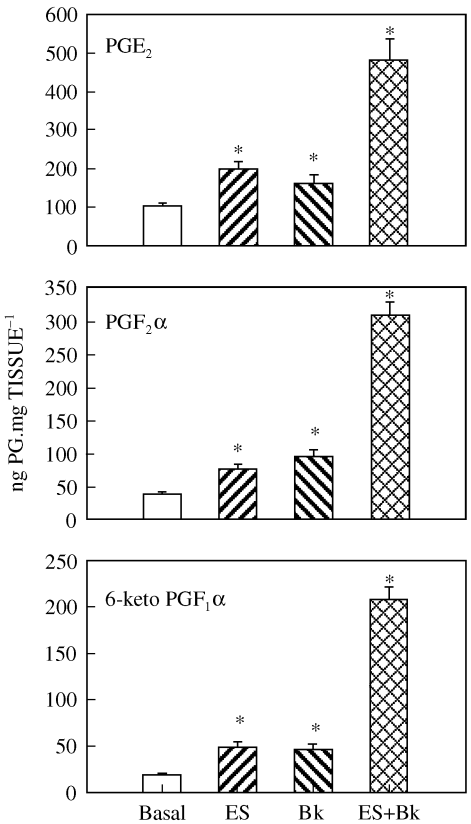

Fig. 1 PG production in the epididymal portion of the vas deferens in resting conditions; (basal): □; during electrical stimulation (0.1 Hz, 1 ms): ▨; in the presence of 1 μM Bk: ▧; and during electrical stimulation in the presence of Bk: ▩. The tissues were incubated for 35 min.
Shown are mean values ± SEM of 5 experiments per group.
*$P<0.05$ when compared to basal values.

RESULTS

The isolated epididymal portion of the rat vas deferens produced and released PGE_2, $PGF_{2\alpha}$ and 6-keto $PGF_{1\alpha}$ (stable metabolite of PGI_2 or prostacyclin) to the incubation medium. Figure 1 shows that the greatest production, expressed as ng.mg tissue^{-1} in 35 min of incubation ($n=7$), was that of PGE_2 (92.5 ± 9.5), followed by $PGF_{2\alpha}$ (36.5 ± 3.1) and 6-keto $PGF_{1\alpha}$ (18.3 ± 1.7). We did not identify other prostanoids.

As shown in Figure 1, electrical stimulation (0.1 Hz, 1 ms) increased the production of the three PGs by approximately 100% as compared to basal values. Similar increases were obtained in tissues exposed to 1.0 μM Bk. When both stimuli, electrical stimulation and Bk, were applied simultaneously, the increases in PG release over the basal values were approximately 1100% for PGE_2, 800% for $PGF_{2\alpha}$ and 400% for 6-keto $PGF_{1\alpha}$.

Figure 2 shows the effects of adrenergic antagonists on the production of prostaglandins induced either by electrical stimulation or by 1.0 μM Bk or by both stimuli applied simultaneously. The α_1 adrenoceptor antagonist prazosin (0.1 μM) abolished the effect of electrical stimulation on the production of the three PGs. Moreover, in tissues pretreated with prazosin, the release of PGs induced by the combination of Bk and electrical stimulation did not differ from the PG production observed with Bk alone. Neither yohimbine (1.0 μM), an α_2 adrenoceptor antagonist, nor propranolol (1.0 μM), a β adrenoceptor antagonist, modified the release of PGs induced by electrical stimulation or by electrical stimulation plus Bk. The adrenoceptor antagonists did not alter either the basal or the Bk-induced production of PGs.

Figure 3 shows the effects of Bk-receptor antagonists on the release of PGs. The selective B_2-receptor antagonist D-Arg-Hyp^3 D-Phe^7 Bk (1.0 μM) abolished the increase in PGs production induced by 1.0 μM Bk, both in non-stimulated and in electrically stimulated tissues. The effects of Bk on PG production were not modified by

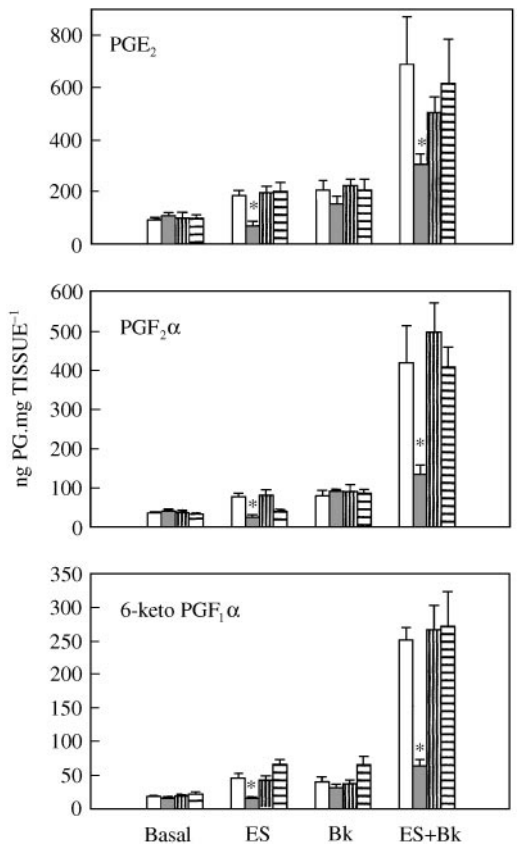

Fig. 2 Effect of adrenoceptor antagonists on the PG production in resting conditions (basal) and during exposure to electrical stimulation (ES), to 1.0 μM Bk or electrical stimulation plus Bk (ES+Bk) in the epididymal portion of the vas deferens. The following adrenoceptor antagonists were present in the organ bath: □ none; ▩ prazosin (0.1 μM); ▥ propanolol (1.0 μM); ▤ yohimbine (1.0 μM).
*$P < 0.05$ when compared to respective control values.

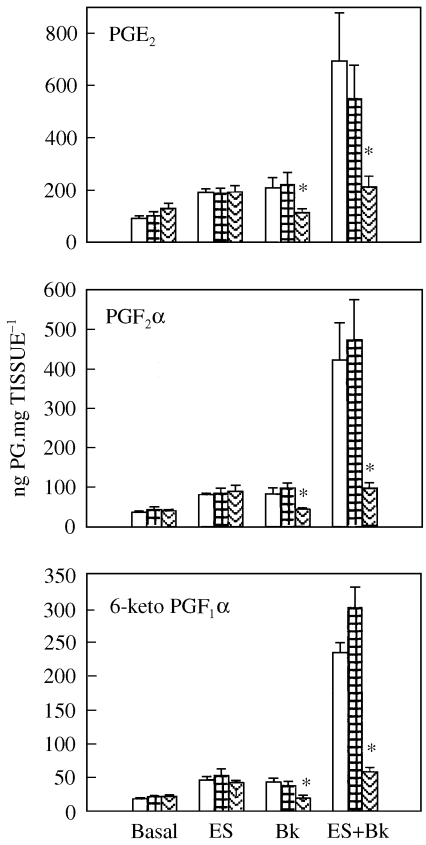

Fig. 3 Effect of Bk receptor antagonists on PG production in resting conditions (basal) and during exposure to electrical stimulation (ES), to Bk (1.0 μM) or electrical stimulation plus Bk (ES+Bk) in the epididymal portion of the vas deferens. The following Bk receptor antagonists were present in the organ bath: □ none; ⊞ des-Arg9, (Leu8)-bradykinin (10.0 μM); ⊠ D-Arg-(Hyp3,D-Phe7) bradykinin (1.0 μM).
*$P < 0.05$ when compared to respective control values.

d-Arg^9 Leu^8 Bk (10.0 μM), a selective B_1-receptor antagonist. These antagonists "per se" did not alter either the basal PG production or the PG production induced by the electrical stimulation.

Effects of Bk on the basal muscle tension and on the twitch responses induced by electrical stimulation

Bk (1.0 μM) elicited contractile responses in non-stimulated as well as in electrically stimulated tissues. These responses did not differ between the two groups (Fig. 4A&4C). Moreover, Bk induced a significant increase in the twitch responses induced by electrical stimulation (Fig. 4C). The PG synthesis inhibitor indomethacin (10.0 μM) did not modify either the contractile response or the increase in the twitches induced by Bk (Figs. 4B–4D).

DISCUSSION

The present study shows that Bk as well as electrical stimulation enhances the production of PGs in the epididymal portion of the rat vas deferens. Moreover, it has been shown that there is a potentiation of the effects when both stimuli are applied simultaneously.

The effects of electrical stimulation herein observed confirm and extend previous findings. Hedqvist and Von Euler[25] reported that the electrically stimulated guinea pig vas deferens releases PGE-like material to the superfusion medium. On the other hand, Peredo and Borda have shown that exposure of rat isolated vasa deferentia to exogenous noradrenaline increases the synthesis and release of PGE_2 through the activation of α adrenoceptors.[5] In this study, we observed that electrical stimulation increased the production of PGI_2 and $PGF_{2\alpha}$ in addition to PGE_2. Moreover, we observed that prazosin

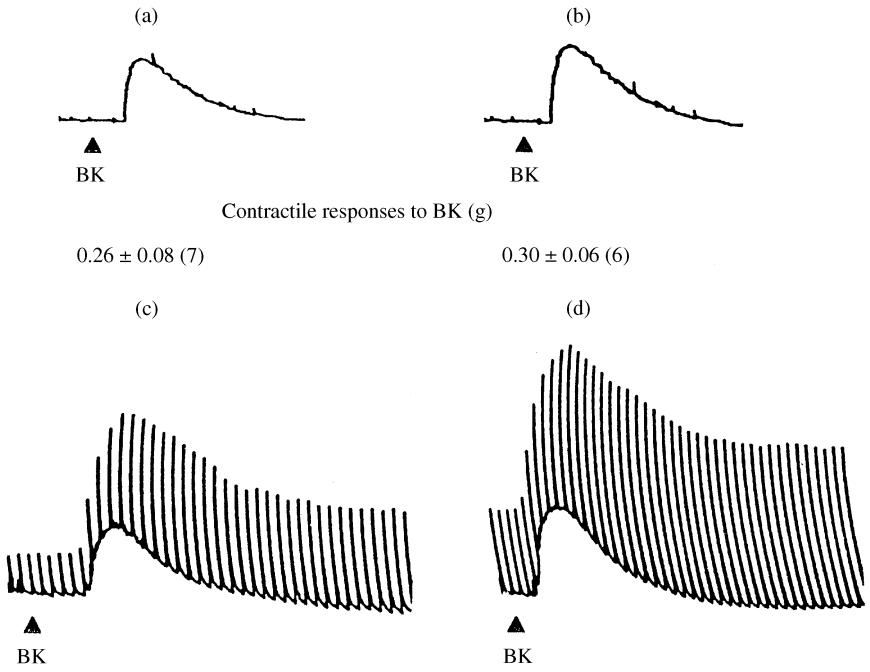

Fig. 4 Effects of Bk on the basal muscle tension and on the twitch responses induced by electrical stimulation (0.1 Hz, 1 ms). The addition of Bk (1.0 μM) to the organ bath to non-stimulated (A and B) and electrically stimulated (C and D) epididymal portions of the vas deferens is indicated by the arrowheads. Indomethacin (10.0 μM, B and D) or its vehicle, (0.1% ethanol, A and C) were added to the organ bath 30 min prior to Bk and remained until the end of the experiment.
Shown are mean values ± SEM. Numbers in parentheses indicate the number of experiments.
*$P < 0.05$ when compared to the twitch responses prior to Bk.

abolished the PG release induced by electrical stimulation, whereas yohimbine and propranolol had no effect. These results suggest that the noradrenaline released by electrical stimulation promotes PG production through the interaction with adrenoceptors of the α_1 subtype and that neither α_2 nor β adrenoceptors are involved.

Bk exerts its effects through the activation of two kinds of receptors, namely B_1 and B_2. The present results suggest that the increase in PGE_2, PGI_2 and $PGF_{2\alpha}$ production induced by Bk in the epidydimal portion of the rat vas deferens is related to the activation of B_2 receptors, since this effect was suppressed in the presence of D-Arg-Hyp^3 D-Phe^7 Bk, a selective B_2 antagonist, whereas it was not modified in the presence of d-Arg^{9}Leu^8 Bk, a selective B_1 antagonist. This is in agreement with previous reports which showed that Bk stimulates eicosanoid production through the activation of B_2 receptors in a number of organs and tissues such as blood vessels,[26] rat uterus,[27] human airways[28] and rabbit isolated perfused ear.[21]

The stimulatory effect of Bk on PG release was greatly increased in electrically-stimulated vasa deferentia when compared with non-stimulated tissues and the effects of Bk combined with field stimulation are greater than those expected from the sum of two independent stimuli. This is particularly evident for PGI_2. The precise mechanism by which this potentiation occurs is not clear at present.

The observation that Bk as well as electrical stimulation, individually or simultaneously applied, increased the release of all the detected PGs to the same extent could suggest that its stimulatory action is exerted at the phospholipase or the cyclooxigenase levels. In support of this view, in the rat perfused heart, Bk has been shown to stimulate phospholipase A_2 and phospholipase C, both of which may provide a source of free arachidonic acid, a rate-limiting-step in the synthesis of eicosanoids.[29] Moreover, α_1 adrenergic stimulation also activates phospholipase A_2 and phospholipase C in FRTL5 thyroid cells.[30]

On the other hand, the fact that indomethacin did not modify the contractile responses either in non-stimulated or in electrically stimulated vas deferens, or the increase in the twitch responses evoked by Bk, suggests that prostanoids are not involved in the mechanism of those contractile effects. This is in accord with the work of Patra et al. who demonstrated that in the rat and human vas deferens the release of PGE_2 and $PGF_{2\alpha}$ is not correlated with contraction.[31]

In conclusion, these findings indicate that, in the rat vas deferens, the increase in prostaglandin production and release and the contractile effects induced by Bk are independent of each other. The effects of Bk on prostaglandin production appears to depend on the activation of B_2 receptors, while the increase in prostaglandin release induced by electrical stimulation, as well as the potentiation of such effects observed when Bk and electrical stimulation were applied simultaneously, should be mediated by the release of noradrenaline and the subsequent activation of α_1 adrenoceptors.

ACKNOWLEDGEMENTS

The technical assistance of Ms. Fernanda De Fino and Ms. Mónica Ferrari is gratefully acknowledged.

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