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A Scorpion Venom Peptide Fraction Induced Prostaglandin Biosynthesis in Guinea Pig Kidneys: Incorporation of ^{14}C-Linoleic Acid

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A peptide fraction isolated from the venom of the Egyptian scorpion *Buthus occitanus* was proved to have a bradykinin- potentiating activity. *In vivo* and *in vitro* modes of action of the isolated bradykinin- potentiating peptide (BPP) on kidneys of guinea pigs were investigated. Animals received five successive i.p. doses of the scorpion BPP (1 μg/g body weight) at one-week intervals. The control animals were i.p. injected with saline solution only. *In vivo* experiments showed a significant increase in renal tissue PGE_2 content and lipid peroxides of the treated guinea pigs compared to the control animals ($p < 0.05$). Nonsignificant changes were detected in the levels of tissue c-AMP and 5-nucleotidase activity ($p > 0.05$) of the treated animals, while the changes in c-GMP and c-AMP/c-GMP ratio were both significant ($p < 0.05$). *In vitro* experiments demonstrated enhanced capacity of guinea pig-renal tissue to convert ^{14}C-linoleic acid to its metabolites, 6-keto-$PGF_1\alpha$, $PGF_2\alpha$, PGE_2, TxB_2, PGD_2, and arachidonic acid, in response to the added PBP (1 μg/ml) and bradykinin (1 μg/ml). This enhanced response was abolished upon the addition of 1 μg/ml of BK-inhibitor (D-Arg- [Hyp^3, Thi^{5,6}, Phe^7]). The capacity for labeled metabolites recovery in BPP treated renal tissue was 19.78%, while it was 13.00% in the basal control. The total increase that evoked by BPP was 62.78%. The results clearly indicate that the isolated BPP induced prostaglandin biosynthesis, which may trigger enhanced glomerular filtration in guinea pigs.

Key words: bradykinin potentiating peptide (BPP), c-AMP, ^{14}C linoleic acid, kidney, lipid peroxides (LPOs), prostaglandin E_2 (PGE_2), scorpion venom.

Abbreviations: BK, bradykinin; BPP, bradykinin potentiating peptide; LPOs, lipid peroxides; PGs, prostaglandins.

Growing interest has recently focused on venom peptide fractions and their possible clinical implications as therapies for several pathomechanisms (*1–3*). The venom of the Egyptian scorpion *Buthus occitanus* was found to contain a peptide fraction with bradykinin-potentiating activity that could remarkably enhance urea and creatinine clearance (*4*). The isolated bradykinin-potentiating peptide (BPP) acted *in vivo* as a growth factor (*5*, *6*), promoting cellular growth response of ovarian follicle and uteri (*7*) and stimulated spermatogenesis in premature mice (*8*).

Some responses to bradykinin (BK) appeared to be mediated by generation of eicosanoids, seemingly as a result of stimulation of phospholipase A_2 (*9*, *10*). It was proposed that a functional coupling of BK and prostaglandins (PGs) within the kidney might be effective in the regulation of renal blood flow and salt-water excretion (*11*, *12*). PGs and BK are known as potent vasodilators and cause the contraction of longitudinal smooth muscle from stomach to colon (*13*). PGs, leukotrienes and related compounds are called eicosanoids because they are derived from 20-carbon essential fatty acids (*13*, *14*). Rose (*15*) claimed that PGs play a local role in preventing an excessive response to anti-diuretic hormone (ADH). Hence, Gray (*16*) reported that PGs modulate the renal actions of ADH. Renal PGs, primarily PGA and PGE, are produced in medulla by the collecting duct epithelium and the medullary interstitial cells (*17*, *18*). After their release, they enter the renal cortex via the ascending limb of Henle and/or the vasa recta, where they are converted into inactive metabolites. An antinatriuretic effect of PGs was suggested by the finding that PGA and PGE augment Na^+ excretion, probably as a result of reduced proximal reabsorption (*19*, *20*). In rabbit kidney medulla, PG synthase catalyzed the formation of PGE_2, $PGF_2\alpha$ and PGD_2 from arachidonic acid (*21*, *22*). A delicate interrelationship exists between angiotensin II (ANG II) and renal PGs in the modulation of renal blood flow, glomerular filtration rate and possibly tubular electrolyte handling (*23*). PGE_2 indirectly alters the tubular Na^+ reabsorption through changes in medullary interstitial tonicity via ADH (*24*). Also, PGE_2 was found to decrease Na^+ transport in isolated collecting ducts of rabbit kidney (*25*, *26*). Other authors (*27*) observed that decreased K^+ concentration enhanced PGE_2 synthesis in renal medullary interstitial cells in culture. It was also reported that rat kidney glomeruli have the capability of synthesizing PGE_2, $PGF_1\alpha$, PGI_2 (6-keto-$PGF_1\alpha$), PGD_2, and TxB_2 from arachidonic acid (*28*, *29*). However, human renal medul-

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lary microsomes have the capacity to synthesize considerably more PGE_2 and related metabolites than cortical microsomes (*30*). Attallah and Lee (*31*) stated that PGE_2 was found to be the major compound produced by renal tubular and interstitial cells.

The kidney possesses two powerful hormonal systems, the rennin-angiotensin system and the prostaglandin system (*32*). The B-adrenergic pathway of rennin stimulation is independent of any modifying influence exerted by PGs. However, the endogenously synthesized PGs can antagonize some of the actions of the renal adrenergic nervous system (*33*). Handler (*34*) found that tubular antidiuretic action of PGE_2 might reflect a direct input on renal tubular adenylate cyclase, resulting in an increase of water permeability. There are both stimulators and inhibitors of PGE_2 receptors in the kidney (*35*). In turn, PGE_2 can exert both stimulatory and inhibitory effects on c-AMP metabolism. Therefore, the increase in the glomerular production of vasodilatory prostanoids was considered to be an important mediator of the increase in glomerular filtration rate (GFR) following an acute protein load (*36*). However, the inhibition of PGE_2 synthesis was found to be associated with a significant fall in creatinine clearance in animals with reduced renal mass (*37*). Nevertheless, the activity of PGs as modulators of renal homodynamic events depends on their interaction with other hormones such as ANG II and arginine vasopressin (AVP) and BK. Podjarny and associates (*38*) reported that a cessation of production of PGs and related substances in the kidney might have a direct impact on the development and maintenance of renal failure. They also found that sodium depletion in the inner medulla was associated with a marked increase in PGE_2 and TxB_2 synthesis. Consequently, this work was conducted to investigate the possible promoting activity of the venom peptide BPP on the biosynthesis of PGs in kidneys of experimental animals that may enhance their glomerular filtration rates.

MATERIALS AND METHODS

(i) Isolation, Purification and Identification of the Venom BPP—A crude venom of the Egyptian scorpion *Buthus occitanus* was kindly donated by Dr. Nassar of Assiut University, Egypt. A peptide with bradykinin potentiating activity was chemically isolated from the venom following the procedure described by Ferreira (*39*) with slight modification. Almost one gram of the crude venom was suspended in 100 ml of distilled water and heated for 5 min in a boiling water bath with 750 ml of ethanol (99%). The mixture was centrifuged for 60 min at 2,000 rpm. The supernatant was evaporated under reduced pressure in a rotatory evaporator. Three successive additions each of 10 ml of 90% ethanol were used to extract the BPP from the dry fraction. To purify the crude BPP, 4 volumes of ethyl ether were added to the alcoholic extract, and the mixture was shaken. The precipitate was separated by centrifugation, disolved in 10 ml of distilled water and dialyzed. The aqueous solution was then lyophilized and stored refrigerated under vacuum. For characterization, 20 μg of the lyophilized fraction was dissolved in about 100 μl of normal saline solution and subjected to electrophoresis on cellulose acetate, Oxoid strips (No. 50 size 160 mm × 25 mm) in sodium phosphate buffer pH 7.7, u = 0.1, 30 V/cm for 12 h. The isolated fractions were characterized on the strips as distinctive zones under UV at 360 nm. For further identification of the BPP as recommended by Kato and Suzuki (*40*), 20 μg of the lyophilized fraction was dissolved in 300 μl of a solvent mixture of absolute ethanol–chloroform–water–saturated *n*-butanol (1:1:1, v/v) and applied to thin layer chromatography. Silica gel 60 F_{254} plates 5 × 20 cm, 0.25 mm thickness (Merck, Darmstadt, FRG) were activated for 1 h before use. TLC was developed in *i*-butanol–acetic aci $–H_2O$ (3:1:1, v/v), then sprayed with 0.4% ninhydrin after drying.

(ii) Bioassay of BK-Potentiating Activity—The pharmacological effect of the isolated venom peptide was demonstrated by an increase in the contraction of the guinea pig ileum in the presence of bradykinin. The increase in the contraction was recorded in the presence of synthetic bradykinin (batch No. B-3259, Sigma Chemical, St. Louis, MO, USA). The maximum contraction response was recorded (by Oscillograph 400 MD_2C, Plamer Bioscience, Washington, USA) 2–20 min after the addition of 0.3 μg of the lyophilized venom BPP per ml of Tyrode's solution. Bradykinin (0.02 μg/ml) was added 50 s after the addition of the venom peptide fraction. The venom BPP was compared with known bradykinin potentiators, B and BPP5a (Sigma Chemical, St. Louis, MO, USA). The potentiating unit (PU) was determined as the concentration of peptide which is able to duplicate the magnitude of contraction produced by a defined dose of Bk on isolated gunia pig ileum (*40*, *41*).

(iii) In Vivo Experiments—Sixteen healthy growing male guinea pigs of about 6 weeks of age and body weight of 250 to 300 g were used for in vivo experiments. All animals were provided with a commercial balanced diet and tap water *ad libitum*. They were housed in plastic cages at natural photobic periods, at room temperature, and allowed to acclimate to the environment prior to experimental use. Animals were divided into two equal groups. One group received five successive intraperitoneal doses of the scorbion BPP (1 μg/g body weight) at one–week intervals. The dose was adapted in accordance with (*8*). The control group received saline solution only in the same way. Immediately after killing the animal by decapitation, the abdomen was opened and the kidney was excised and weighed. A 1% tissue homogenate of kidney was prepared in 100 mM phosphate buffer, pH 7.4 containing 22 mg% EDTA and 1.0 mg% indomethacin to prevent further processing of PG biosynthesis. Tissue homogenates were stored at –20°C until analysis for PGE_2, cyclic nucleotides, lipid peroxides and 5-nucleotidase.

(iv) Analytical Techniques—Determination of prostaglandins: PGE_2 was extracted from kidney-tissue homogenates in accordance with Lands and Smith (*42*). Its content was assayed as described by Lingren *et al.* (*43*) using the PGE_2 [^{3}H]RIA Kit (Cat. No. 600001, Advanced Magnetic, Cambridge, MA, USA). PGE_2 counting rates were correlated with concentrations by a standard curve set experimentally as described by Jaffe *et al.* (*44*).

Determination of c-AMP and c-GMP: Before assaying c-AMP and c-GMP by RIA, tissue cycling nucleotides extracts were prepared following the protocol of Steiner (*45*). c-AMP was measured using Incstar RIA ^{125}I-c-AMP-

Kit (Cat. No. 74065, Incstar Corporation, Stillwater, MN, USA). c-GMP was assayed by RIA ^{125}I-c-GMP-Kit (Cat. No. 6302, Advanced Magnetics, Cambridge, MA, USA). Labeled analyte counts were correlated with concentrations by means of a calibration curve that was developed experimentally as indicated by Harper and Brooker (*46*).

Determination of 5-nucleotidase activity: 5-Nucleotidase activity was measured in kidney-tissue homogenate using the method of El-Aaser and El-Merzabani (*47*). The enzyme activity was calculated as μM inorganic phosphorus (P_i)/min/mg protein in the tissue homogenate.

Determination of lipid peroxidation: Levels of lipid peroxides (LPOs) were measured in tissue homogenates as described by El-Saadani *et al.* (*48*) with little modifications. Briefly, 100 μl of tissue homogenate was diluted with 0.01 M phosphate buffered saline, pH 7.4 to a final volume of 1 ml. Then 100 μl of the diluted homogenate was vortex–mixed with 5 μl of EDTA (24 μM), 2 μl of BHT (20 μM), and 1 ml of the color reagent (catalogue No. 14106, Merck, Darmstadt, FRG). The mixture was let stand for 30 min in the dark at ambient temperature, then the absorbance (*A*) at 365 nm was measured in a 1-cm quartz cuvette against reagent blank. The concentration was calculated using the equation: LPOs (nM/ml) = 110.7 *A*/0.0246, based on the molar absorptivity of I_3 (24,600 M^{-1} cm^{-1}) at 365 nm, and the dilution factor of the homogenate. To exclude the possible interference in the reagent blank reading, the background was corrected by measuring LPOs against the immediate mix without the 30 min incubation.

(v) In Vitro Experiments—Kidneys of 24 of the same guinea pigs used for *in vivo* experiments were excised immediately after sacrifice as described by Lennon and Poyer (*49*). Three-percent tissue homogenates of the pooled kidneys were made in ice-cold Krebs-Ringer bicarbonate buffer containing 119 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl, 1.2 mM KH_2PO_4, 15 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1 mg/ml of glucose, and 1 mg/ml of bovine serum albumin with a gas phase of 95% O_2–5% CO_2. Tissue homogenate was then divided to four equal volumes in separate flasks: control, treated with BPP (1 μg/ml), treated with BPP and BK (1 μg/ml for each), treated with BPP and BK-inhibitor (D-Arg-[Hyp^3, Thi^{5,6}, Phe^7]) (1 μg/ml for each). Each of these four volumes was subdivided into two equal parts for the duplication of the readings. All flasks were incubated for 50 min at 37°C to activate tissue enzymes, then 10 μl of ^{14}C-linoleic acid containing 159.54 × 10^4 dpm (specific activity 52 mci/mM, Amersham, England) was added to each. Enzymatic conversions of linoleic acid into arachidonic acid (AA), prostaglandins (PGs), and thromboxanes (Txs) were stopped after 30 min. Flasks were placed onto ice-bath and the pH of the mixture was lowered to 3–4 by adding 1.0 ml of saline followed by 100 μl of 1.0 N HCl to each container. ^{14}C-linoleic acid metabolites were extracted by shaking twice with two volumes of ethyl acetate. The organic solvent extracts were pooled, recentrifuged to remove tissue particles, and the supernatant was evaporated under nitrogen. The residue of each extract was dissolved in 100 μl of chloroform/methanol mixture (1:3, v/v) to be applied for chromatographic separation (*50*).

(vi) Resolution of ^{14}C-Linoleic Acid Metabolites by TLC—The extracted metabolites were applied as spots on TLC plates and separated by a one-dimensional technique (*51*, *52*). The solvent used was ethylacetate–isooctane–acetic acid–water (11:5:2:10, v/v). Reference standards of arachidonic acid, prostaglandins, and thromboxanes were used. The developed spots of the standard and samples were visualized by spraying with 10% phosphomolybdic acid in ethanol, and each spot was scraped off into a separate vial. Prostaglandins and thromboxane were extracted with methanol, to which 3 ml of scintillation solution of toluene/ triton X-100, 2:1, v/v containing 7.5 g PPO/liter was added. The radioactivity was counted by liquid scintillator counter (ISOCAP/300 liquid scintillation system, Searle Analytic) and the recovery rates were determined for each of the labeled compounds. Counting efficiency of samples was 60–70% as determined by means of a ^{14}C-calibration curve that was developed experimentally (Fig. 1).

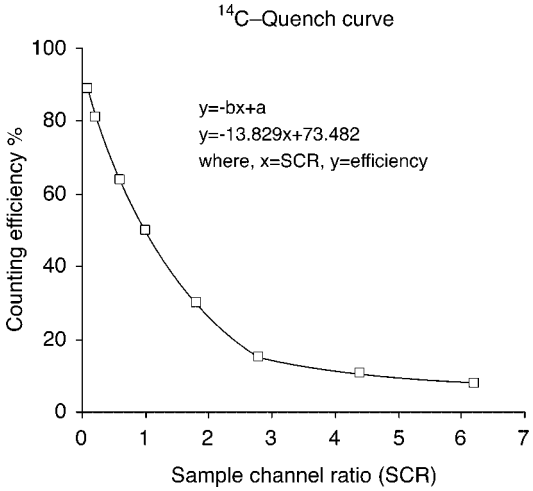

Fig. 1. **^{14}C-calibration curve.**

(vii) Statistical Analysis—Statistical analyses were performed by an analysis of variance to determine statistical significance among the groups and by student's *t*-test to determine significance between groups. Result were considered statistically significant at $p < 0.05$.

RESULTS

The isolated peptide fraction of the *Buthus occitanus* venom (BPP) was proved to have bradykinin–potentiating activity. However, it showed less potent BK potentiation on guinea pig ileum than both potentiator B and

Table 1. **Bioassay for BK potentiating activity.** *In vitro* potentiation of BK activity on isolated guinea pig ileum was assayed as described in the experimental section. The potentiating unit (PU) is the concentration of peptide which is able to duplicate the magnitude of contraction produced by a defined dose of Bk on isolated gunia pig ileum.

Peptide fraction	PU (μg/ml)
BPP-B	2.40 ± 0.90
BPP_{5a}	1.80 ± 0.60
Isolated scorpion BPP	120 ± 2.80

Results are mean ± SEM (n = 6).

Table 2. ***In vivo* effects of *Buthus occitanus* BPP on levels of PGE_2, LPOs, cyclic nucleotides, and 5-nucleotidase activity in renal tissue of male guinea pigs.**

Parameter	Control	BPP
PGE_2 (ng/100 mg wet tissue)	73.91 ± 5.60	103.76 ± 6.12*
LPOs (nM/mg wet tissue)	3.53 ± 0.46	6.38 ± 0.59*
c-AMP (pM/ 100 mg wet tissue)	147.34 ± 7.10	141.93 ± 8.09
c-GMP (pM/ 100 mg wet tissue)	7.65 ± 1.56	10.91 ± 2.09*
c-AMP/c-GMP	19.26	13.01*
5-Nucleotidase (μM P_i/min/100 mg wet tissue)	9.73 ± 0.51	8.64 ± 0.42

Values are mean ± SEM. Samples are measured in duplicates. Each value represents the mean of 16 readings (n = 16). *$p < 0.05$.

BPP_{5a} (Table 1). The BK-potentiating unit of the tested venom BPP on the isolated guinea pig ileum was 120 ± 2.80 μg/ml. BPP did not show sensitizing activity or detectable bradykinin-like activity on the muscle preparation.

In vivo experiments showed a significant increase in renal tissue PGE_2 content of the treated guinea pigs compared to the control animals ($p < 0.05$, Table 2). Concomitantly, LPOs levels were significantly increased ($p < 0.05$) in treated animals. Nonsignificant changes were detected in the levels of tissue c-AMP and 5-nucleotidase activity ($p > 0.05$) of the treated animals, while the changes in c-GMP and c-AMP/c-GMP ratio were both significant ($p < 0.05$, Table 2).

In vitro experiments demonstrated the capacity of guinea pig-renal tissue to convert ^{14}C-linoleic acid enzymatically to its metabolites. These labeled metabolites, 6-keto-$PGF_1\alpha$, $PGF_2\alpha$, PGE_2, TxB_2, PGD_2, and arachidonic acid, were detected at different levels following each treatment (Table 3). In renal tissue treated with BPP or BPP plus BK, the total conversion into labeled metabolites was markedly increased. However, the treatment with BPP plus BK-inhibitor did not show any significant change compared to the basal control (Table 3). The recovery of radioactivity in metabolites of the BPP-treated group was 19.78% while, it was 13.00% in the control one. The total increase evoked by BPP was 62.78%. This ratio was even higher in arachidonic acid fraction (193.30%), and in PGD_2 it was 58.21%. The increase in the recovered metabolites in the group treated with BPP plus BK was 100.86% for the total and 70.18% for 6-keto-$PGF_1\alpha$, 94.61% for $PGF_2\alpha$, 119.48% for PGE_2, 79.58% for TxB_2, and 181.20% for PGD_2 compared to the control. While kidney tissue treated with BPP plus BK-inhibitor showed a recovery of 14.51%, the increase in the arachidonic acid fraction was 104.20%.

DISCUSSION

Several peptides isolated from animal venoms have been shown to potentiate BK action on smooth muscles by at least two different mechanisms: by sensitization of BK receptors or by inhibiting the angiotensin-converting enzyme (*53*). The tested scorpion BPP was previously shown to potentiate BK in different mammals in both ways (*4*, *7*, *8*). In most of the mammalian tissues investigated, prostaglandin synthetase activity was found (*54*). Mammalian cells metabolize arachidonic acid *via* cyclooxygenase and lipoxygenase pathways, which result in various prostaglandins, thromboxanes, hydroxy fatty acids, leukotrienes and lipoxins (*55*, *56*). For instance, BK and thrombin were found to stimulate the conversion of exogenous arachidonate to PGs, implying enhancement of cyclooxygenase activity (*57*). In kidney mesangial cells, PGE_2 is selectively stimulated by ANG II and AVP (*58*). Both glomerular epithelial and mesangial cells have receptors for ANG II and AVP, and these receptors are tightly linked to intracellular pools of phospholipase, cyclooxygenase and PGE_2 isomerase to increase PGE_2 (*58*). The response of some isolated organs to BK is enhanced by some of the bioactive components in venoms of snakes and scorpions, which are called Bk-potentiating peptides or fractions, BPP or BPF (*59*). Bk has been shown to release PGs in a variety of tissues including rabbit's kidney (*12*, *60*), cat's spleen (*61*), rat's heart (*62*) and isolated rat kidney (*63*).

In vivo experiments showed that the BPP isolated from scorpion *Buthus occitanus* venom significantly elevated the PGE_2 in kidney of the successively treated animals (Table 1). The present results are consistent with the report by Nasjletti and Malik (*64*) that Bk promoted PG biosynthesis by selective activation of phospholipase A_2. This in turn enhanced the conversion of arachidonic acid

Table 3. ***In vitro* effect of *Buthus occitanus* BPP on ^{14}C-linoleic acid metabolism in renal tissue of guinea pigs.** (Radioactivity: dpm × 10^4).

Metabolite	Control	BPP	BPP + BK	BPP + BK-ihibitor
6-Keto-$PGF_1\alpha$	1.14 ± 0.24	1.32 ± 0.14	1.94 ± 0.55	1.06 ± 0.27
$PGF_2\alpha$	1.67 ± 0.36	1.86 ± 0.52	3.25 ± 0.94*	1.68 ± 0.48
PGE_2	3.08 ± 0.73	5.67 ± 0.91*	6.76 ± 1.18*	2.60 ± 0.76
TxB_2	5.78 ± 1.06	5.71 ± 1.23	10.38 ± 1.57*	4.50 ± 1.58
PGD_2	5.48 ± 0.83	8.67 ± 1.92	15.41 ± 2.26*	5.98 ± 1.02
A.A.	3.59 ± 1.16	10.53 ± 2.05*	3.92 ± 1.37	7.33 ± 1.94
Total	20.74 ± 4.38	33.76 ± 6.77	41.66 ± 7.87	23.15 ± 6.05
Recovery (%)	13.00	19.78	25.63	14.51

Values are mean ± SEM. Each value represents the mean of 8 readings (n = 8). *$p < 0.05$ *versus* control groups.

into PGs by cyclooxygenase, various PG endoperoxide isomerases, and reductase (*65*). Jesse and Cohen (*66*) stated that BK stimulated PG synthesis by activating phospholipase A_2, which is responsible for releasing PG precursors from membrane phospholipids. However, phospholipase A_2 activity could be enhanced by the hydroxyl radical ($OH^{\cdot}$), which would result in arachidonic acid release and the generation of various endoperoxide metabolites (*67*, *68*). Interestingly, the present results demonstrated that LPOs as a lipid peroxidation marker were significantly increased ($p < 0.05$) in the kidneys of BPP-treated animals. Other authors mentioned that lipid peroxidation processes lead to enhanced increase in PGs level, which is mainly mediated through cyclooxygenases and other oxidases (*69–72*). Furthermore, the level of lipid peroxides is a key control of cyclooxygenase activity, the enzyme associated with a shift of arachidonic profile towards the production of PGs (*73*, *74*).

The present *in vivo* experiments also showed no significant change in the levels of c-AMP in renal tissue of treated animals compared to the control. In contrast c-GMP levels were significantly increased after the successive exposure to the venom BPP. Generally, a delicate interrelation exists between renal PGs and the modulation of renal blood flow, GFR and possibly tubular electrolyte handling (*75*). Basically, most tissues possess two major classes of receptors for controlling cellular functions and proliferation. One class triggers the production of c-AMP, while the other induces inositol phospholipid turnover, frequent arachidonic acid release and c-GMP, but not c-AMP, production (*76*). Furthermore, free radicals lead to a decrease in adenylate cyclase activity, which is phospholipid-dependent and causes a decrease in tissue c-AMP. Oxygen increased guanidylic cyclase and tissue c-GMP (*77*). It was also reported that enhanced levels of tissue c-GMP could be attributed to guanylate cyclase activity, which is stimulated by polyunsaturated fatty acids with specific determinants, and to peroxidases, lipid peroxides and oxygen free radicals (*78*). In addition, Zenser *et al.* (*79*) claimed that physiological effect of BK on renal function might be mediated by c-GMP and PGs. This was exactly the case in the present investigation: the successive injection of the venom BPP resulted in a significant increase in both renal tissue PGE_2 and c-GMP. PGE_2 and c-GMP are fractions known to stimulate the glomerular filtration rate and renal blood flow, as reported by Colina-Chourioetal (*12*) and Levine *et al.* (*36*). Therefore, it could be concluded that the isolated BPP triggered an enhanced glomerular filtration rate and renal blood flow in guinea pigs. In a preliminary histopathology experiment, microscopical examination of semithin sections from kidneys of BPP treated guinea pigs showed a remarkable dilatation of the glomerular blood capillaries (data not shown). This was observed only in the treated animals and could be simply attributed to increased renal blood flow as a result of the injected venom BPP.

The bioactive peptide AVP was found to stimulate PGE_2 synthesis in rat renal medullary epithelial cells independently from the activation of adenylate cyclase (*80*). However, the controversial response in levels of c-AMP and c-GMP was cited by other authors (*81*). Steiner *et al*. (*82*) reported that while a hormonal stimulus caused a marked increase in c-AMP, it did not affect the level of c-GMP, which indicates a separate individual control of these nucleotides. Basically, a change in the ratio of c-AMP/c-GMP signals cell division and hence replication (*77*). Interestingly, Curtis-Prior (*83*) reported that most, if not all the actions of PGs in biological systems are mediated by changing the levels of cyclic nucleotides. Consistent with the present results, it was demonstrated that in bovine and canine veins, the c-AMP/c-GMP ratio was decreased under the influence of PGE_2 (*84*).

Cyclic nucleotides are rapidly metabolized by a tissue-specific phosphodiesterase to 5-AMP (*78*). The present results demonstrate that injection of the isolated BPP caused a nonsignificant decrease in renal tissue 5-nucleotidase activity. In this regard, it is rather difficult to propose a reasonable interpretation. However, Amer and Mckinney (*85*) claimed that PGs may have a direct effect on intracellular phosphodiesterase. Nevertheless, the effect of phosphodiesterase inhibitors on BK-stimulators that increase PGE_2 synthesis by renal medulla are distinct from their effects on c-AMP metabolism (*86*), which may hold for the present results.

It has been postulated that kidney medulla PG-synthase catalyzes the formation of PGE_2 and $PGF_2\alpha$ from arachidonic acid to perform its biological functions (*87*, *88*), which was more or less confirmed by the present *in vivo* results. The *in vitro* experiments predicted such a PG-synthase system in tissues of guinea pig kidney. This enzyme system controled the biosynthesis of radioactive arachidonic acid, PGD_2, TxB_2, PGE_2, $PGF_2\alpha$, and 6-keto-$PGF_1\alpha$ from labelled ^{14}C-linoleic acid added to tissue homogenates (13% in control animals, Table 3). Consistent with this, other investigators detected the same fractions in isolated rat kidney glomeruli (*29*) and sheep seminal vesicular gland microsomes (*89*) after adding ^{14}C-arachidonic acid. The present data show that the added BPP increased the formation of arachidonic acid and its oxygenated fractions (19.78%, Table 3), although the increase was not significant ($p > 0.05$). However, the association of BK with its potentiator BPP showed a significant increase (25.63%, $p < 0.05$). This might simply mean that BPP potentiated the exogenous BK and hence stimulated the biosynthesis of arachidonic acid and its metabolites in renal tissues of guinea pigs. This notion was confirmed by the fact that the increase was diminished by addition of BK-inhibitor plus BPP. In this case, the recovery amounted to only 14.51%. Consequently, it could be concluded that BPP performed its potentiating activity on both endogenous and exogenous BK for PGs biosynthesis. Consistent with the present results, Zusman and Keiser (*27*) postulated that bioactive polypeptides such as BK, AVP, and ANG II increase the release of ^{14}C-arachidonic acid from renal medulla. It is known that PG biosynthesis in the kidney occurs as a result of arachidonic acid release from membrane phospholipids after the activation of phospholipase (*24*). However, PG biosynthesis is controlled in two ways: stimulation of phospholipase A_2 and stimulation of PG synthetase (*89*). So, it appears from the present results that either BK alone or BK potentiated by the isolated venom BPP enhanced the conversion of ^{14}C-linoleic acid to ^{14}C-arachidonic acid and other labeled metabolites, PGD_2, TxB_2, PGE_2, $PGF_2\alpha$, and 6-keto-$PGF_1\alpha$. This was clearly illustrated by the

assessment of radioactivity of identified arachidonic acid spots, in which an increase of 193.30% was observed in treated tissue homogenate of guinea pig kidneys. However, the marked increase of PGE_2 biosynthesis *in vitro* as a response to BPP plus BK can interpret and confirm that recorded *in vivo* experiments. In conclusion, the isolated BPP exerted *in vivo* and *in vitro* effects that resulted in a significant increase in PGE_2 and associated metabolites that might trigger enhanced glomerular filtration and hence improved renal function of guinea pigs. Thus, clinical application of the isolated BPP as a therapy in cases of renal failure should be considered for further investigations.

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