Inhibitory action of bradykinin on release of the adrenergic transmitter in the isolated lapine kidney

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Summary. In the isolated lapine kidney perfused with tyrode solution and prelabeled with [^{3}H] norepinephrine, bradykinin (10 ng/ml) decreased the overflow of tritium elicited by sympathetic nerve stimulation both in the presence and absence of indomethacin. These observations indicate that bradykinin acts at presynaptic sites reducing release of the adrenergic transmitter in the isolated lapine kidney.

Recently we reported that bradykinin inhibits the vasoconstriction produced by norepinephrine and by sympathetic nerve stimulation in the isolated lapine kidney perfused with tyrode solution, and that this effect was diminished during inhibition of prostaglandin synthesis[2]. Inasmuch as bradykinin had a greater inhibitory effect on the renal vasoconstrictor response elicited by nerve stimulation than on that elicited by norepinephrine, we concluded that the peptide inhibits adrenergic transmission by interferring with both pre- and postsynaptic events. The present study was designed to investigate the effect of bradykinin on the release of adrenergic transmitter in the isolated lapine kidney perfused with tyrode solution. The studies were performed in the absence and in the presence of an inhibitor of prostaglandin synthesis, indomethacin, to distinguish those actions of bradykinin that are related to prostaglandin synthesis from those which are not. Thus, any modification of the effect of bradykinin during inhibition of prostaglandin synthesis would indicate participation of a prostaglandin-mediated component.

Methods. Experiments were performed on male New Zealand white rabbits, weighing 2.5–3.0 kg. The animals were anesthetized with sodium pentobarbital (20 mg/kg i.v.) and their abdomen was opened by a midline incision. The aorta was ligated below and above the renal artery, a polyethylene cannula was immediately inserted into the renal artery, and the kidney was flushed with heparinized saline (100 units/ml). The kidney was isolated with the renal vein and the ureter intact and placed in a chamber maintained at 37 °C. The kidney was perfused with tyrode solution at a constant rate of 8 ml/min by means of a Harvard peristaltic pump (model 1210)[3]. A tyrode solution of the following composition (mM) was used: NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.1, $NaHCO_3$ 12, NaH_2PO_4 0.42 and D(+) glucose 5.6. The temperature of this solution was maintained at 37 °C.

Experimental protocol. 40 μCi (18.2 nmoles) of [^{3}H] norepinephrine was infused into the kidney over a period of 20 min to label the endogenous stores of neurotransmitter. The kidney was then perfused with [^{3}H] norepinephrine free tyrode solution for 1 h to wash the radioactivity from extraneuronal spaces. The sympathetic nerve plexus was stimulated at 10-min intervals for 1 min at 4 Hz with supramaximal biphasic rectangular pulses of 1-msec duration by means of a bipoplar platinum electrode (Grass Stimulator S_{44}). The perfusate was collected for 1 min both before and during sympathetic nerve stimulation and the radioactivity was determined in 1-ml aliquots mixed with 10 ml of Insta-Gel emulsifier (Packard Instruments Company) and expressed as cpm per ml of perfusate. Frame and Hedqvist[4] showed that the increase in the ouput of radioactivity caused by stimulation of sympathetic nerves in the isolated perfused kidney of rabbit is mainly due to efflux of intact [^{3}H] norepinephrine. To calculate simulation-induced tritium overflow, the amount of radioactivity released spontaneously prior to nerve stimulation was substracted from the amount of radioactivity obtained during stimulation. The overflow of tritium caused by renal nerve stimulation was determined before (1st period), during (2nd period), and after (3rd period) infusion of either vehicle or bradykinin (10 ng/ml) in kidneys perfused with and without indomethacin, 1 ng/ml. The selection of the kinin concentration was based on our previous work showing that bradykinin at 10 ng/ml increased the release of PGE-like material from the rabbit kidney by about 8-fold[2], which effect was inhibited by indomethacin. The infusion of bradykinin began 6 min before stimulation. To normalize the data we calculated the ratio of both the basal and the stimulation-induced efflux of radioactivity in the 2nd period, during infusion of vehicle or bradykinin, to the corresponding effluxes obtained without the kinin in the 1st and 3rd period (P_2/P_1 and P_2/P_3).

The following drugs were used: (−) norepinephrine [^{3}H] (sp.act. 2.2 Ci/mmole, New England Nuclear). Indomethacin was obtained from Sigma and the synthetic bradykinin diacetate used was purchased from Protein Research Foundation, Minoh-Shi. Indomethacin was dissolved in tyrode solution. Bradykinin diacetate was dissolved in saline and then added to the perfusion medium.

Data are expressed as means ± SE. Student's unpaired t-test was used to compare the difference between the control and experimental groups according to the procedures described by Steel and Torrie[5]. P-values of less than 0.05 were considered significant.

Table 1. Effect of bradykinin (10 ng/ml) on the basal (B) and stimulation-induced (S) overflow of tritium in the isolated lapine kidney perfused with tyrode solution

	n	Radioactivity (cpm/ml)			Ratios	
		1st period (P_1)	2nd period (P_2)	3rd period (P_3)	P_2/P_1	P_2/P_3
	5	Control	Vehicle	Recovery		
B		919 ± 43	901 ± 39	830 ± 16	0.98 ± 0.01	1.08 ± 0.03
S		5332 ± 171	5184 ± 162[a,b]	4872 ± 101		
Δ (S-B)		4413 ± 130	4283 ± 125[a,b]	4042 ± 86	0.97 ± 0.00	1.06 ± 0.01
	5	Control	Bradykinin	Recovery		
B		1070 ± 169	1049 ± 105[b]	953 ± 110	0.98 ± 0.01	1.10 ± 0.03
S		5411 ± 564	4866 ± 474[a]	4920 ± 441		
Δ (S-B)		4341 ± 496	3817 ± 402[a]	3967 ± 383	0.87 ± 0.01[c]	0.96 ± 0.02[d]

Data are expressed as means ± SE; n = number of kidneys. [a] Different from 1st-period value ($p < 0.05$, paired Student's t-test). [b] Different from 3rd-period value ($p < 0.05$, paired Student's t-test). [c] Significantly different from the corresponding value in the vehicle group (probability level, non-paired Student's t-test; $cp < 0.01$).

Table 2. Effect of bradykinin (10 ng/ml) on the basal (B) and stimulation-induced (S) overflow of tritium in the isolated lapine kidney perfused with tyrode solution containing indomethacin (1 µg/ml)

	n	Radioactivity (cpm/ml)			Ratios	
		1st period (P_1)	2nd period (P_2)	3rd period (P_3)	P_2/P_1	P_2/P_3
	7	Control	Vehicle	Recovery		
B		933± 40	899± 33[b]	841± 24	0.96±0.01	1.06±0.01
S		5098±296	5215±313[a]	5220±314		
Δ (S-B)		4165±300	4316±311[a,b]	4379±312	1.03±0.00	0.98±0.00
	6	Control	Bradykinin	Recovery		
B		895±121	875±122[b]	831±125	0.97±0.01	1.05±0.02
S		5081±486	4902±500[a]	4974±511		
Δ (S-B)		4186±425	4027±434[a,b]	4147±449	0.96±0.01[c]	0.97±0.01

Data are expressed as mean±SE; n = number of kidneys. [a] Different from 1st-period value ($p<0.05$, paired Student's t-test). [b] Different from 3rd-period value ($p<0.05$, paired Student's t-test). [c,d] Significantly different from the corresponding values in the vehicle group (probability level, non-paired Student's t-test: $cp<0.01$, $dp<0.05$).

Results. In control experiments, without bradykinin infusion, stimulation of renal nerves at 4 Hz increased the overflow of tritium but this increment decreased slightly during successive periods of stimulation (table 1). During bradykinin infusion (10 ng/ml) electrical stimulation of renal nerves also increased overflow of radioactivity (table 1), but the ratios (P_2/P_1 and P_2/P_3 were lower than the corresponding values obtained during infusion of vehicle only ($p < 0.01$ and $p < 0.05$ respectively) suggesting an inhibitory effect, albeit small, of the kinin on the overflow of tritium caused by sympathetic nerve stimulation (table 1). The ratios of basal efflux of radioactivity, P_2/P_1 and P_2/P_3, were not affected by bradykinin. Similar results were obtained in the kidney perfused with tyrode solution containing indomethacin (1 µg/ml); the overflow of tritium produced by renal nerve stimulation was reduced by bradykinin as was the P_2/P_1 but not the P_2/P_3 ratio of tritium overflow (table 2). The P_2/P_1 ratio of stimulation-induced tritium overflow during bradykinin infusion was greater in the presence (0.96±0.01) than in the absence (0.87±0.01) of indomethacin ($p < 0.01$). However, the P_2/P_1 ratio of stimulation-induced tritium overflow during infusion of vehicle only was also greater in the presence (1.03±0.00) than in the absence (0.97±0.00) of the prostaglandin synthesis inhibitor ($p < 0.001$).

Discussion. In the isolated lapine kidney prelabeled with [^{3}H] norepinephrine, stimulation of sympathetic nerves increases the output of tritiated products, primarily that of intact [^{3}H] norepinephrine[4]. The present study demonstrates that bradykinin reduced the stimulation-induced overflow of tritium suggesting a presynaptic action of the peptide to inhibit release of adrenergic transmitter in the lapine kidney. This is in accord with a report that bradykinin inhibits release of norepinephrine in the isolated rabbit heart and pulmonary artery[6]. In contrast, the kinin did not affect release of the neurotransmitter in the isolated perfused canine spleen[7]. The effect of bradykinin in reducing release of the adrenergic transmitter in the isolated rabbit heart and pulmonary artery was reduced by inhibitors of cyclooxygenase suggesting involvement of prostaglandins. In the isolated perfused lapine kidney, bradykinin selectively stimulates synthesis of PGE_2, a prostaglandin that inhibits norepinephrine release caused by sympathetic nerve stimulation[8–10]. That the P_2/P_1 ratio of stimulation-induced tritium overflow during bradykinin infusion in the present study was greater in the presence than in the absence of indomethacin is consistent with contribution of prostaglandins to the inhibitory action of the kinin on transmitter release. However, the observation that the P_2/P_1 ratio of stimulation-induced tritium overflow during infusion of bradykinin vehicle was also greater in the presence than in the absence of indomethacin precludes such an interpretation. Thus, participation of prostaglandins in the inhibitory action of bradykinin on norepinephrine release in the lapine kidney is uncertain.

Previous work on the effect of bradykinin on vascular reactivity to sympathetic nerve stimulation and exogenous norepinephrine in the rabbit[2] and dog kidney[11] indicated that the kinin acts at postsynaptic sites to reduce adrenergically-induced vasoconstriction. However, the possibility of an additional effect of the peptide interferring with release of norepinephrine was suggested by the observation in the isolated lapine kidney that the kinin had a greater inhibitory effect on the vasoconstrictor response to nerve stimulation than on that elicited by injected norepinephrine[2]. In this context, the results of the present study suggest that an action of bradykinin at presynaptic sites may contribute to the peptide effect in reducing the vasoconstriction caused by nerve stimulation.

That bradykinin inhibits release of the adrenergic transmitter and reduces vascular reactivity to norepinephrine suggests that the kinin modulates adrenergic transmission by interferring with both pre- and postsynaptic events at the neuroeffector junction in the kidney. The effective concentration of bradykinin, 10 ng/ml, is several fold greater than that reported in arterial blood[12], which argues against a physiological role of circulating kinins in modulation of renal vascular neurotransmission. However, substantial amounts of kinin, lysyl-bradykinin, are generated continuously within the kidney by a kallikrein occurring in the renal cortex[12]. Therefore, it is conceivable that the renal kallikrein-kinin system contributes to modulates adrenergic transmission in the kidney.

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