Original Article

The involvement of bradykinin in adrenaline-induced pulmonary edema in rats

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We investigated the effect of endogenous bradykinin on adrenaline-induced pulmonary edema (PE) by blocking bradykinin receptors. In preliminary experiments, a bolus injection of adrenaline (ADR; 10 μg/kg) solution (10 μg/ml) was determined to be an edematogenic dose for inducing PE. The lung body weight index (LBI) and incidence of PE (IPE) were determined. The IPE and LBI of the group pretreated with Des-Arg^9-[Leu^8]-Bradykinin (DA-BK, 50 μg/kg, 50 μg/ml) increased significantly compared with those of the control group ($p<0.05$). On the other hand, there were no remarkable changes in IPE and LBI in the groups pretreated with Hoe140 (D-Arginyl-[Hyp^3, Thi^5, D-Tic^7, Oic^8]-Bradykinin (100 μg/kg, 100 μg/ml), captopril (20 mg/kg, 20 mg/ml) or L-NAME (1 mg/kg 1 mg/ml). Moreover, the IPE and LBI of the group co-treated with L-NAME and DA-BK decreased compared with the DA-BK group ($p<0.05$). Thus, bradykinin aggravates adrenaline-induced PE through activation of the B$_2$ receptor by the kallikreins as a result of the ADR administration, although the precise mechanism is not known.

Key words: Adrenaline, pulmonary edema, endogenous bradykinin, B$_2$ receptors

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Introduction

Despite the remarkable progress made in the field of anesthesiology and the medicine of resuscitation there are still some reports of adrenaline-induced pulmonary edema (ADR-induced PE) in man. Two cases were recently reported in which patients developed ADR-induced PE after local injection of ADR for hemostasis. Many interpretations have been offered to explain ADR-induced PE: however none of them can be accepted as conclusive. This problem has for many years attracted the attention of investigators, not only because of its peculiar pharmacological aspects but also because of the possible analogy with acute pulmonary edema in man. Most studies to date on ADR-induced PE have concentrated principally on determining the effects of some hemodynamic conditions[1].

Although it cannot be denied that mechanical elements play an important role in the condition, i.e. hypertension in the pulmonary and general circulation, it appears unlikely that mechanical factors alone can account for the production of ADR-induced PE. For this reason some investigators have directed their attention to the existence of other factors that may participate in the process, such as permeability of the vascular walls in the lung, which might facilitate or determine the mechanical effects of hypertension. It can be assumed that drugs which increase capillary permeability in the lung may play an important role in the etiology of ADR-induced PE[2]. With regard to histamine and serotonin, there is already strong evidence for such a role, however, as far as we are aware, there has been little work regarding the involvement of endogenous bradykinin (eBK) in ADR-induced PE. Because kinins are potent stimulators of the release of nitric oxide

(NO), we also investigated whether NO is involved in ADR -induced PE. From these standpoints we performed a three -phase study: 1) the effects of BK receptor activation in ADR-induced PE were examined. 2) B_2 receptor activation by eBK after administration of ADR was determined. 3) the possibility of nitric oxide (NO) as a participating factor was examined.

Material and methods

General procedures

All animal handing techniques were carried out according to the guiding principles governing the care and use of laboratory animals approved by The Japanese Pharmacological Society. Male Wistar rats (n = 88) weighing 250 to 300 g (Sankyo Laboratories, Japan) were housed under controlled conditions for at least 2 wk prior to the use in the experiments. The rats were randomly divided into 11 groups of 8 rats each. Each rat was anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg) and then fixed on its back. After tracheal incubation with a polyethylene tube, the femoral vein and artery were catheterized to provide routes for drug administration and to monitor the arterial blood pressure through a blood pressure transducer (E10-PZ, Nihon Kohden, Tokyo, Japan), respectively. For monitoring respiratory movement, a thermister connected to a low pressure transducer (AA-601H Nihon Kohden,Tokyo, Japan) was attached to the polyethylene tube in the trachea. The body temperature of the rats was maintained at approximately 38.5°C during the experiment.

Test drugs

The following drugs were used: adrenaline (Bosmin; Daiichiseiyaku, Co., Ltd., Tokyo, Japan). Phentolamine-mesylate (Regitin; CIBA-GEIGY Co. Ltd, Takarazuka, Japan). Hoe140 (D-Arginyl-[Hyp[3],Thi[5],D-Tic[7],Oic[8]]-Bradykinin, a selective B_2 receptor antagonist; Peptide Institute,Inc., Osaka, Japan). DA-BK (Des-Arg[9]-[Leu[8]]-Bradykinin; a selective B_1 receptor antagonist; Peptide Institute, Inc. Osaka, Japan). Captopril (Sigma Chemical Co., St. Louis, MO, America). L-NAME (N[G]-Nitro-L-arginine Methyl Ester Hydrochloride; Wako Pure Chemical Industries, Ltd. Osaka, Japan). All drugs were prepared daily, dissolved in 0.9% NaCl , and protected from light.

Determination of the edematogenic dose of ADR

ADR was diluted in saline at a concentration of 10 μg/ml (pH 6) before use. A bolus dose of ADR (10 μg/kg) solution was administered through the venous cannula. The rats were killed by drawing blood 20 min after administration of ADR to examine the lungs. The lungs were rapidly removed, and the attached tissues were trimmed away on gross observation. A 0.2 - ml sample of blood was obtained once before ADR administration and twice after ADR administration to determine alterations in Pco_2 and Po_2 using a Blood Gas System (ABL5; Radiometer Co., Copenhagen, Denmark). The net weight of the lungs (NWL) was measured to evaluate the degree of PE. The development of PE was judged by 1) macroscopic hemorrhage observed as hemorrhagic patches and spots in the lung and 2) froth or liquid running out of the tracheal tube. The lung was considered not to have PE only when no froth came out from the removed lower trachea even when the lung was gently squeezed[3]. The lung body weight index (LBI = lung weight $\times$ 100 / body weight) was calculated in every rat to quantify the degree of excess fluid retention in the lung. The incidence of PE (IPE) was also calculated in every group. The area under the BP-time curve (AUC) values was determined by subtracting the BP value obtained prior to ADR administration (baseline BP) from the systolic BP obtained following ADR injection. The BP values were recorded every min while systolic BP remained above the baseline BP value[4]. The values were subsequently integrated and the value obtained defined as AUC. BP values below the baseline BP were regarded as zero.

Effect of pretreatments with DA-BK, Hoe140, captopril, and L-NAME

Rats (n = 48) were subdivided into 6 groups of 8 rats each. Three min or ten min prior to injecting of the edematogenic dose of ADR, bolus doses of DA-BK (50 μg/kg), Hoe140 (100 μg/kg), captopril (20 mg/kg) , or L-NAME (1 mg/kg), and combinations of these drugs (DA-BK + Hoe140, and L-NAME + Hoe140) were administered to rats in various groups. In each rat, the development of pulmonary edema was assessed according to the same procedure described above.

Effect of co-treatment with phentolamine and DA-BK

Phentolamine (0.3 mg/Kg) was administered to 8 rats 10 min before ADR administration. In another group of 8 rats phentolamine and DA-BK were administered 10 min and 3 min, respectively, prior to ADR administration. The condition of the lungs was investigated and compared with the group pretreated with phentolamine alone.

Effect of co-treatment with phentolamine and Hoe 140

In a group of 8 rats phentolamine and Hoe 140 were administered 10 min and 3 min, respectively, prior to ADR administration. The condition of the lungs was investigated and compared with the group pretreated with phentolamine alone.

Effect of co-treatment with DA-BK and L-NAME

L-NAME and DA-BK were administered to 8 rats 10 min prior to ADR administration. The lungs were examined as described above, and compared with the group pretreated with DA-BK alone.

Statistical analysis

Data are expressed as the mean± SD. Statistically significant differences among groups were determined using the two-tailed Student's t-test, and the statistical analysis of IPE was carried out by the chi-square test. A P value of less than 0.05 was considered to be statistically significant.

Results

Effect of adrenaline

In the preliminary study, 10 μg/kg or more of ADR successfully induced PE. We thereby selected 10 μg/kg ADR as the edematogenic dose. ADR administration produced a sustained rise in systolic and diastolic BP, followed by a discontinuous drop in BP, and usually induced short bouts of respiratory distress throughout the period of observation (Fig.1). The systolic blood pressure increased from 120± 4 to 250± 14 (mmHg). The following parameters were changed in the control group by ADR administration: the AUC: 162± 47 (mm^2), the LBI: 0.5± 0.1, the IPE: 50% (Table), Pco$_2$: 54± 15 , Po$_2$: 81± 15 (mmHg) (Table).

Effect of DA-BK

Intravenous injection of DA-BK 3 min before ADR administration did not exhibit remarkable changes of the systolic blood pressure, AUC, or respiratory movement. Nevertheless, in animals pretreated with DA-BK, ADR strikingly increased the LBI from 0.5± 0.1 to 0.64± 0.14 (P<0.05) , the IPE from 50% to 100% (P<0.05), and decreased the blood gas (Po$_2$) from 81± 15 to 58± 20 (P<0.05). Therefore, pretreatment with DA-BK increased the severity of ADR-induced PE.

Effect of combination of DA-BK and Hoe140

After intravenous injection of a combination of DA-BK and Hoe140, administration of ADR increased the AUC from 162± 47 to 268± 68, but did not exhibit remarkable changes of the systolic BP (244± 9), the LBI (0.46± 0.05), or the IPE (62.5%) compared with those of the control group. The LBI and the IPE decreased compared with these of the group pretreated with DA-BK. These decreases were statistically significant (P<0.05) (Table).

Effect of Hoe140, captopril, and L-NAME

In the rats pretreated with Hoe140, the IPE and the LBI seemed to be increased from 50% to 75%, and from 0.50± 0.10 to 0.60± 0.14 respectively, but these increases were not statistically significant. In the group pretreated with L-NAME, there were no differences in any parameters, and only the AUC of captopril group was increased (P<0.05) (Table).

Effect of co-treatment with phentolamine and DA-BK

Pretreatment with a combination of phentolamine and DA-BK increased the LBI from 0.34± 0.02 to 0.37± 0.04 and the IPE from 0% to 37.5% compared with the group pretreated with phentolamine alone (Table). The increase in the LBI was not significant, however the net weight of the lung increased (P<0.05) (Table).

*Effect of co-treatment with phentolamine and Hoe 140*

Pretreatment with a combination of phentolamine and Hoe140 did not change the LBI, the IPE and the net weight of lung compared with the group pretreated with phentolamine alone (Table).

Effect of co-treatment of L-NAME and DA-BK

Aggravation of ADR-induced PE in the group treated with DA-BK was reversed by pretreatment with L-NAME. The LBI was reduced from 0.64± 0.14 to 0.49± 0.08 (P <0.05) (Table).

Discussion

The results of this study demonstrate that eBK aggravates ADR-induced PE, which is mediated *via* stimulation of the B$_2$ receptors. Pretreatment with the selective B$_1$ receptor antagonist DA-BK increased the severity of ADR-induced PE, while Hoe140, a selective B$_2$ receptor antagonist can convert the effect. There were no significant differences between the means of

DA-BK   ADR
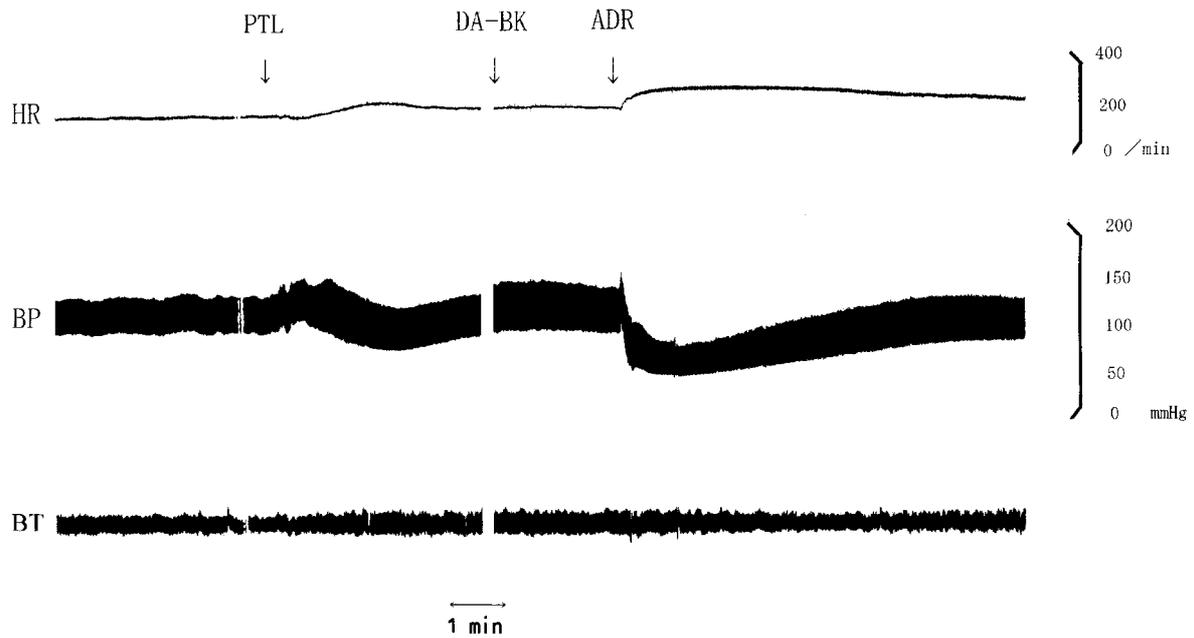
↓   ↓

HR

BP

BT

←——→
1 min
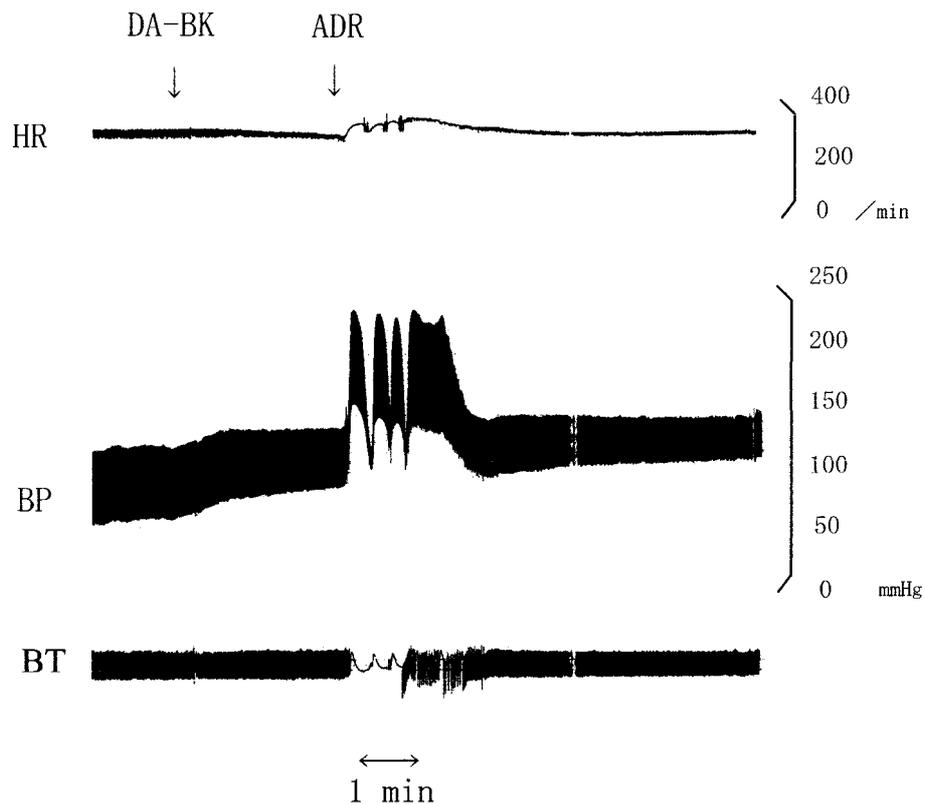
Fig. 1. Typical record of the hemodynamic and breathing changes after ADR injection in animals treated with DA-BK. The abbreviations are follows: heart rate (HR); systemic blood pressure (BP); breathing (BT).

Table   Effect of pretreatment with various drugs on ADR-induced PE

Material group	Systolic BP (mmHg)			AUC(mm²)	LBI	IPE (%)
n=8	before pretreat[1]	before ADR[2]	after ADR[3]			
Control	—	120±4	250±14	162±47	0.50±0.10	50
Hoe140	115±9	117±8	236±13	169±45	0.6±0.14	75
DA-BK	116±5	117±7	245±10	187±30	0.64±0.14*	100
DA-BK+Hoe140	115±7	115±11	244±9	268±68	0.46±0.05**	62.5
Captopril	121±4	101±8	230±14	254±62	0.46±0.1	50
L-NAME	115±10	142±11	255±4	174±18	0.53±0.19	62.5
L-NAME+Hoe140	120±8	135±6	256±7	178±8	0.54±0.05	62.5
L-NAME+DA-BK	114±7	146±9	247±9	204±58	0.49±0.08***	50

Systolic BPs were measured 1) just before each pretreatment and 2) just before ADR administration. Peak values of systolic BP were obtained 3) after ADR administration. AUC was calculated over 5 min. Abbreviations are as follows: blood pressure(BP) adrenaline(ADR) lung body weight index(LBI), area under the BP-time curve(AUC), and incidence of pulmonary edema(IPE). Significant difference (P<0.05) by the unpaired t-test. The statistical analysis of IPE was carried out by the chi-square test. *Significant difference (P<0.05) from control group. **Significant difference (P<0.05) from group treated with DA-BK. ***Significant difference (P<0.05) from group treated with DA-BK.

Fig. 2. Typical records of the hemodynamic and breathing changes after ADR injection in animals treated with phentolarnine and DA-BK. The abbreviations are follows: heart rate (HR); systemic blood pressure (BP); breathing (BT); phentolamine (PTL), Des-Arg^9- [Leu^8] -Bradykinin (DA-BK); adrenaline (ADR).

Table   Effect of DA-BK on PTL pretreatment in ADR-induced PE

treatment group	Systolic BP(mmHg)		LBI	NWL(g)	IPE(%)
n=8	before treatment[1]	after ADR [2]			
PTL	128±10	64±7	0.34±0.02	0.95±0.03	0
PTL + DA−BK	123±9	60±7	0.37±0.04	1.10±0.1	37.5
PTL+Hoe 140	124±11	72±6	0.34±0.01	0.96±0.04	0

Data are expressed as the mean± SD. Systolic BPs were measured 1) just before each pretreatment and 2) after ADR administration. Abbreviations are as follows: phentolamine(PTL), adrenaline(ADR), Des-Arg^9-[Leu^8]-Bradykinin (DA-BK), blood pressure(BP), lung body weight index(LBI), the net weight of the lung(NWL), and incidence of pulmonary edema(IPE).

the AUC of the group pretreated with DA-BK and the control group. Similarly, there were no significant differences between the group pretreated with DA-BK and that co-treated with L-NAME and DA-BK except LBI and IPE. The onset mechanism of ADR-induced PE is primarily hydrostasis, therefore there were no obvious changes in the hemodynamic parameters such as the systolic BP and the AUC in our experiment. These results suggest that the pretreatments with DA-BK or

Hoe140 did not affect the development of PE via the same mechanisms involved in hemodynamic PE and that increased vascular permeability is a facilitating factor.

Kinin might be generated by kallikrein originating from the arterial wall and/or myocardium. Kallikrein mRNA is present in vascular tissue and myocardium and kallikrein is synthesized and released from these regions[5]. In addition, kininogen and the kallikrein

Table   Effect of DA-BK on the change of Pco_2 and Po_2 in ADR-induced PE

group	$Pco_{2(mmHg)}$		$Po_{2(mmHg)}$	
n=8	before ADR[1]	after ADR[2]	before ADR[1]	after ADR[2]
control	37±4	54±15	102±11	81±15
DA−BK	41±6	69±17	103±13	58±20*

Data are expressed as the mean± SD. Pressure of blood CO_2 and blood O_2 were measured. 1) just before ADR administration. 2) after ADR administration. Abbreviations are as follows: adrenaline (ADR); pressure of blood carbon dioxide (Pco_2); pressure of blood oxygen (Po_2); Des-Arg[9]-[Leu[8]]-Bradykinin (DA-BK). *Significant difference (P<0.05) compared with control group by the unpaired t-test.

products (*i.e.*, BK and double-chain kininogen) are present in both vascular tissue and myocardium[6,7]. Thus, eBK can be rapidly produced in the circulatory system in the presence of stimulant factors. High doses of ADR induce conspicuous consumption of circulatory rat kininogen. Kininogen consumption by ADR is accompanied by a rise in rat plasma toluenesulphonylarginine methyl ester (TAME) esterase which is attributed to the activation of plasma kallikrein by pro-kininogenase generated in circulatory basophils or mast cells exposed to ADR[8]. Thus, an ample dose of ADR induces the production of eBK in the circulatory system. Bradykinin increases vascular permeability *via* B_2 receptors in a variety of organs[9-12]. The fact that BK-induced plasma extravasation is inhibited by Hoe140 provides further evidence for the importance of B_2 receptors in producing acute plasma extravasation[13]. The severity of PE in the group pretreated with DA-BK was alleviated by Hoe140. Reports that BK induces plasma extravasation by acting on B_2 receptors support our findings.

Phentolamine was administrated in order to evaluate eBK's action on B_1 receptors and B_2 receptors on PE under the condition blocking α-receptors .The LBI and the IPE of the group pretreated with phentolamine and DA-BK were not different compared with those of phentolamine group. However, the NWL of the group pretreated with phentolamine and DA-BK was increased. Pretreatment of phentolamine and Hoe 140 did not change the NWL. These data suggest also that B_1 receptors antagonist aggravated the severity of ADR-induced PE.

An angiotension-converting enzyme inhibitor leads to BK accumulation by reducing BK breakdown[14,15]. In the present study, the severity of ADR-induced PE was not affected by increasing eBK by pretreatment with captopril. When the eBK acts on B_1 receptors as well as B_2 receptors simultaneously in the group of captopril pretreatment and control group, the severity of PE was not

changed compared with that of the group pretreated with DA-BK and Hoe140. The differences in the LBI and the IPE of the group pretreated with Hoe140 were not statistically significant compared with these of the control group. Furthermore, the pretreatment of DA-BK aggravated the severity of PE compared with that of control group. These results also suggest a possibility that B_1 receptors have a protective action against ADR-induced PE.

Nitric oxide (NO), a free radical gas implicated in a wide variety of biologic reactions, is a novel signaling molecule that might regulate vasodilation, blood flow, and vascular permeability. Kinins are potent stimulators of NO release; that is, increasing eBK will lead to NO release. It is possible that BK stimulates NO release directly from the endothelium. Studies indicate that NO can be synthesized and released from the endothelium[16,17]. It is also possible that BK acts on cells other than endothelium to stimulate the production of NO. Studies indicate that NO can be produced by macrophages, platelets, and neutrophils[18-20]. Thus, it is possible that BK stimulates NO release from these cell types. In the present study, to confirm the inhibitory effect of the NOS inhibitor, L-NAME, on permeability, L-NAME was administered to the animals immediately before administration of DA-BK and ADR. Pretreatment with L-NAME alone did not alter the severity of ADR-induced PE. Pretreatment with L-NAME, however, reversed the aggravation effect of DA-BK on ADR-induced PE. As discussed above, DA-BK aggravates ADR-induced PE by increasing the microvascular permeability to eBK which acts on the B_2 receptors. The selective increase in permeability of pulmonary microvessels in the group pretreated with DA-BK was significantly inhibited by L-NAME. These results suggest that NO and NOS are involved in the alteration of vascular permeability for activation of B_2 receptors on ADR-induced PE. Extensive evidence suggests that the increase in selective permeability in microvessels is

mediated by NO[21,22]. The fact that the microvessel permeability increase mediated by NO is enhanced by BK infusion[21] strongly supports our results. The mechanism by which BK and NO modulate protein and fluid leakage has not yet been definitively established. The involvement of blood flow modulation in the increase in microvascular permeability is a matter of controversy. Many possible factors contribute to PE, including an increase in microvascular permeability and an increase in blood flow.

We hypothesize two possible mechanisms for the effect of BK on microvessels. First, BK might bind to B_2 receptors on the vessel tissue surface, activating NOS and initiating NO production. As a result, the production and release of NO increase permeability in lung capillaries. Second, because of the blood vessel dilatation effect of NO, the lung blood flow modulation might result in increased microvascular permeability.

In conclusion, our findings suggest that eBK acting on the B_2 receptors increases the severity of ADR-induced PE through an increase in pulmonary vessel permeability and the production and release of NO. At the same time we are in the opinion that B_1 receptor might play an important role actively to protect the rat from ADR-induced PE.

Acknowledgments

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