Br. J. Pharmacol. (1993), **108**, 318–322

Protective effects of bradykinin on the ischaemic heart: implication of the B_1 receptor

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1 We studied the role of bradykinin (BK) and its active metabolite Des-Arg^9-BK on noradrenaline release in association with the incidence of ventricular arrhythmias at reperfusion of the ischaemic myocardium.

2 Experiments were performed in Langendorff perfused isolated hearts of rats subjected to 30 min no flow followed by 5 min reperfusion. The electrocardiogram was monitored continuously and noradrenaline was measured in the effluent as well as in the myocardial tissue.

3 In untreated hearts, cumulative noradrenaline overflow following global ischaemia reached 226 ± 35 pmol g^{-1} of heart ($n = 8$, $P < 0.05$) during the 5 min of reperfusion along with ventricular tachycardia and/or fibrillation. A decrease in myocardial noradrenaline (-31%) was also observed.

4 Bradykinin perfused at concentrations between 0.01 and 1 μM, 10 min before flow was stopped and at reperfusion, inhibited noradrenaline overflow in a concentration-dependent manner. At a concentration of 1 μM, bradykinin completely abolished noradrenaline overflow. For the same concentration of bradykinin, myocardial noradrenaline contents were significantly higher ($n = 5-8$, $P < 0.05$). Ventricular fibrillation but not ventricular tachycardia was also prevented.

5 Des-Arg^9-BK (0.1 μM) in the same experimental conditions had similar effects. While Hoe 140, a selective antagonist at B_2 receptors, did not abolish the effects of bradykinin, Lys [Leu^8] Des-Arg^9-BK, an antagonist at B_1 receptors, abolished the effects of both Des-Arg^9-BK and bradykinin.

6 These results suggest that the cardioprotective action of bradykinin in the preparation may be mediated partially by an inhibitory effect on noradrenaline liberation which could be mediated by the activation of B_1 receptors.

Keywords: Myocardial ischaemia-reperfusion; bradykinin; Des-Arg^9-BK; noradrenaline; arrhythmias; Hoe 140; Lys [Leu^8] Des-Arg^9-BK

Introduction

Myocardial ischaemia is defined as an insufficient blood supply to cardiac tissue. If the blood supply is not restored promptly, the affected tissue will die. Reperfusion is thus the most important way to salvage the ischaemic tissue. However, reperfusion causes damage such as morphological alterations, increased resting tension, increased enzyme leakage (Hearse, 1977; Jennings *et al.*, 1981) and it can also precipitate severe ventricular arrhythmias, including lethal ventricular fibrillation (Corr & Witkowski, 1983).

The sympathetic nervous system is potentially involved in reperfusion-induced arrhythmias. In fact, reperfusion is accompanied by a massive liberation of noradrenaline (NA) into the coronary effluent, the amount of which is probably related to the occurrence of sustained ventricular tachycardia (Godin *et al.*, 1985).

Bradykinin (BK), a well known vasodilator peptide via B_1 and B_2 receptors (Regoli & Barabe, 1980) has been shown to be effective against ischaemia/reperfusion injuries. Its cardioprotective effects have been attributed to improvement in cardiac performance, preservation of high energy rich phosphate, abolition of reperfusion arrhythmias (Linz *et al.*, 1990), as well as an increase in nutritional flow across the capillary wall increasing thereby glucose uptake and utilization (Rösen *et al.*, 1983). A reduction of noradrenaline overflow (Carlsson & Abrahamsson, 1989), and an increase in prostacyclin and nitric oxide release (Van Gilst *et al.*, 1987; Palmer *et al.*, 1987) are other potential effects of bradykinin on the ischaemic phenomenon.

Des-Arg^9-BK, the active metabolite of bradykinin and the typical B_1 receptor agonist (Regoli & Barabe, 1980) has been shown to exhibit some significant effects on vascular tone (Churchill & Ward, 1986; Deblois & Marceau, 1987). However, normal tissues do not generally respond to Des-Arg^9-BK (Regoli & Barabe, 1980). In fact, different authors have shown the induction of this B_1 receptor in pathological circumstances such as tissue trauma, inflammation or anoxia (Bouthillier *et al.*, 1987; Marceau & Regoli, 1991).

In the present study we have shown that B_1 receptors could be involved in the cardioprotective action of bradykinin in rat isolated hearts submitted to reperfusion after acute global ischaemia.

Methods

Perfusion procedure

Male Wistar rats (250–275 g) were anaesthetized with diethylether and 200 iu of heparin was injected intravenously. Hearts were removed rapidly and perfused according to the Langendorff mode at a constant pressure of 100 cmH_2O with a modified Krebs-Henseleit (KH) solution continuously gassed with O_2/CO_2 (95:5) at a temperature of 37°C as previously described (Chahine *et al.*, 1991). The composition (mM) of the solution was: NaCl 118.7, KCl 1.8, $CaCl_2$ 3, $MgSO_4$ 0.85, KH_2PO_4 1.2, $NaHCO_3$ 25, ascorbic acid 0.057, EDTA 0.027, and glucose 5.5. The low concentrations of K^+ and Mg^{2+} and high Ca^{2+} were chosen for their contribution to increase arrhythmias (Lubbe *et al.*, 1978; Keren *et al.*, 1988).

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Global ischaemia was produced by interrupting aortic flow for 30 min followed by 5 min reperfusion. During the stop flow period, hearts were superfused with the same KH solution to prevent an important temperature drop. Under such conditions, hearts continue to contract very slowly and do not stop beating completely. The epicardial electrogram (EPI-ECG) and heart rate were recorded on a Grass polygraph model 50 with 2 electrodes positioned, one above the aorta and the other at the apex of the heart.

Quantification of arrhythmias

Ventricular fibrillation (VF) was assessed when the ECG recording showed chaotic activity with an amplitude less than that of the normal ECG. When at least six consecutive rapid regular beats occurred, this was considered as ventricular tachycardia (VT).

Experimental protocol

Hearts were allowed to equilibrate for 15 min. (i) In the control group (C; $n = 5$), they were perfused for 35 min without any intervention. (ii) In the ischaemic group (ISC; $n = 15$) a stop flow of 30 min was followed by 5 min of normal reperfusion. (iii) In the treated groups ($n = 6$ to 10), KH solution containing bradykinin (1 to 0.01 μM) or Des-Arg^9-BK (0.1 μM) was added to the perfusion medium 10 min before stop flow and continued to the end of the experiment. The maximum dose of Des-Arg^9-BK was 0.1 μM since a higher concentration of this peptide induced depression of heart contractility. (iv) In two groups ($n = 8$ each), Hoe 140 (1 μM) or Lys [Leu^8] Des-Arg^9-BK (1 μM), the antagonists of B_2 and B_1 receptors respectively, were perfused 10 min before bradykinin (0.1 μM) and remained in the tissue to the end of the experiment. (v) In the last group ($n = 8$) Lys[Leu^8] Des-Arg^9-BK was perfused 10 min before Des-Arg^9-BK (0.1 μM) and to the end of the experiment. ECG was monitored continuously. The effluent perfusate was collected before ischaemia and at reperfusion for determination of coronary flow and biochemical parameters. At the end of the experiments hearts were frozen at − 80°C until noradrenaline content determination.

Biochemical assays

Noradrenaline output Samples for catecholamine assay were collected from the coronary effluent before stop flow and during the 5 min reperfusion. The samples (1 ml each) were preserved on ice, immediately stabilized by the addition of a preservation solution (0.025 mM EGTA + 0.02 mM glutathione) and stored at − 80°C until assay. A radioenzymatic determination of noradrenaline was performed (Peuler & Johnson, 1977) after testing the absence of cross reactivity of all pharmacological agents used. Results were corrected by coronary flow and heart weight.

Myocardial noradrenaline was determined by high performance liquid chromatography (h.p.l.c.) using about 100 mg of myocardial tissue from heart apex according to the method previously described (Chahine *et al.*, 1991) and the amounts expressed as nmol mg^{-1} protein after the determination of heart protein content according to Lowry *et al.* (1951).

Bradykinin was also quantified in the perfusate fluid by a radioimmunoassay (RIA) method. Briefly, Tyr^8 BK was iodinated with Na ^{125}I (IMS 30, Amersham) and purified by h.p.l.c. on a ODS C18 column using a linear gradient of acetonitrile in formic acid. RIA was performed as previously described (Adam *et al.*, 1989) using a rabbit polyclonal antibody specific to the carboxy terminal Arg; the sensitivity of the method was 6 pg ml^{-1}.

Drugs

All the compounds of the Krebs-Henseleit solution, bradykinin and Des-Arg^9-bradykinin were purchased from Sigma (St. Louis, MO, U.S.A.). Hoe 140 (D-Arg-[Hyp^3,Thi^5,D-Tic^7, Oic] bradykinin) was generously supplied by Hoechst AG, Germany and Lys [Leu^8] Des-Arg^9-BK was prepared in Dr D. Regoli's laboratory. All solutions were freshly prepared on the day of experimentation.

Statistics

To assess the incidence of VF and VT as well as to compare the two events, Fisher's exact test was used. Results are expressed as mean ± s.e.mean. Statistical significance between groups was determined by Dunnett's test. In all cases, a $P < 0.05$ was considered to be significant.

Results

Arrhythmias and cardiodynamics

After stopping flow, all ischaemic untreated hearts developed bradycardia; furthermore, between 20 and 30 min sino-atrial and high degree AV block were also noted. At reperfusion, VF and/or VT were observed (Table 1). In these hearts, coronary flow decreased by only 20% after 5 min reperfusion (Table 2). In the bradykinin pretreated hearts, a concentration of 1 μM abolished sino-atrial and AV block completely, but not bradycardia, during the stop flow period; at this concentration bradykinin completely prevented the development of VF at reperfusion (Table 1). A slight decrease in heart rate (data not shown) and a slight increase in coronary flow were observed immediately after bradykinin (1 μM) perfusion and remained moderate for 10 min. While Hoe 140 1 μM was not able to antagonize the protective effect of 0.1 μM bradykinin against VF, Lys [Leu^8] Des-Arg^9-BK seemed to abolish partially the effects of bradykinin. Des-Arg^9-BK (0.1 μM) in the same experimental conditions significantly diminished the incidence of VF (Table 1); however, a slight decrease in coronary flow was observed (Table 2). Lys [Leu^8] Des-Arg^9-BK was effective in antagonizing the effects of Des-Arg^9-BK. It is important to mention that antagonists alone reduced the coronary flow slightly, however, they had no influence on the incidence of arrhythmias.

Biochemical parameters

Noradrenaline overflow In control hearts there was a basal release of noradrenaline (3.6 ± 0.22 pmol min^{-1} g^{-1} of heart) which did not change significantly after 35 min of normal perfusion. In untreated hearts submitted to 30 min stop flow,

Table 1 Effects of bradykinin (BK) and Des-Arg^9-BK on the incidence of ventricular tachycardia (VT) and fibrillation (VG) at reperfusion post ischaemia in the absence and presence of antagonists

	VT	*VF*
Control	0	0
Ischaemia	15/15	13/15
BK 0.01 μM	5/6	5/6
BK 0.1 μM	7/10	4/10*
BK 1 μM	4/6	0/6*
Hoe 140 1 μM + BK 0.1 μM	6/8	3/8*
Lys [Leu^8] Des-Arg^9-BK 1 μM + BK 0.1 μM	7/8	6/8
Des-Arg^9-BK 0.1 μM	5/8	3/8*
Lys [Leu^8] Des-Arg^9-BK 1 μM + Des-Arg^9-BK 0.1 μM	7/8	7/8

Control: hearts not submitted to ischaemia.
*$P < 0.05$ compared to ischaemia.

Table 2 Coronary flow (ml min^{-1}) in control, ischaemic untreated and treated hearts

	− 20	− 1	2	4	5 (min)
Control	15.2 ± 1.4	15.0 ± 1.34	–	–	14.5 ± 1.25
Ischaemia	15.0 ± 1.2	15.2 ± 1.36	12.8 ± 2.50	12.5 ± 2.80	12.0 ± 1.7*
BK 0.01 μM	14.8 ± 1.70	14.6 ± 0.98	13.2 ± 1.52	13.0 ± 1.40	11.8 ± 1.00*
BK 0.1 μM	14.5 ± 0.80	14.4 ± 0.86	13.3 ± 0.66	12.5 ± 0.75	12.5 ± 0.87
BK 1 μM	14.4 ± 0.75	15.8 ± 1.04	14.6 ± 1.24	13.8 ± 1.20	13.0 ± 1.04
Hoe 140 1 μM + BK 0.1 μM	15.2 ± 1.57	13.8 ± 1.60	12.7 ± 0.97	12.5 ± 0.85	12.7 ± 0.96
Lys [Leu^8] Des-Arg^9-BK 1 μM + BK 0.1 μM	16.1 ± 1.0	14.0 ± 1.14	12.1 ± 1.12*	11.5 ± 0.98*	11.0 ± 0.88*
Des-Arg^9-BK 0.1 μM	15.3 ± 1.60	13.9 ± 1.83	12.8 ± 2.00	12.5 ± 1.55	11.8 ± 1.22*
Lys [Leu^8] Des-Arg^9-BK 1 μM + Des-Arg^9-BK 0.1 μM	15.8 ± 0.95	14.3 ± 1.12	12.3 ± 1.00*	11.4 ± 0.90*	10.8 ± 1.05*

− 20: before drug administration; − 1: before 30 min stop flow; 2, 4, and 5 min reperfusion.
*$P<0.05$ compared with the corresponding basal value at − 20 min, $n = 5-8$.
BK: bradykinin; Hoe 140: B_2 antagonist; Lys [Leu^8] Des-Arg^9-BK: B_1 antagonist.

noradrenaline overflow reached 226 ± 35 pmol g^{-1} during the 5 min of reperfusion (Figure 1). Pretreatment with bradykinin caused a concentration-dependent decrease in the ischaemia-induced release of noradrenaline at reperfusion with a complete abolition at 1 μM. Des-Arg^9-BK (0.1 μM) also reduced noradrenaline overflow to 80% of the control value. Antagonists (Hoe 140 and Lys [Leu^8] Des-Arg^9-BK) given alone before agonists, had no effect on noradrenaline release; only the latter blocked the protective effects of Des-Arg^9-BK as well as partially those of bradykinin.

Myocardial noradrenaline Tissue noradrenaline stocks were reduced to 31% in ischaemic untreated hearts. Pretreatment with 1 μM bradykinin preserved the myocardial noradrenaline content and to a lesser extent this effect was observed also with 0.1 μM bradykinin and 0.1 μM Des-Arg^9-BK; the antagonists were inactive on their own and partially prevented the effects of the kinins (Figure 2).

Bradykinin As shown in Table 3, the concentrations of bradykinin measured in the coronary effluent were significantly lower than the original concentrations added to the KH buffer solution. A recovery between 25 and 50% of the initial value was observed.

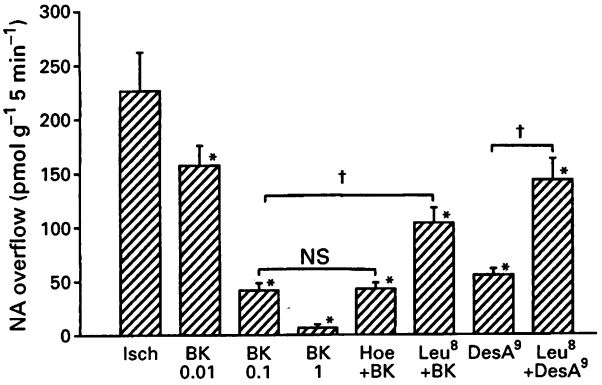

Figure 1 Cumulative noradrenaline (NA) overflow occuring above the basal release during 5 min reperfusion following 30 min stop flow in Isch: ischaemic untreated hearts; BK 0.01: ischaemic hearts pretreated with 0.01 μM bradykinin; BK 0.1: pretreated with 0.1 μM bradykinin; BK 1: pretreated with 1 μM bradykinin; Hoe + BK: ischaemic hearts pretreated with 1 μM Hoe 140 before 0.1 μM bradykinin; Leu^8 BK: pretreated with Lys [Leu^8] Des-Arg^9-BK 1 μM before 0.1 μM bradykinin; DesA^9: ischaemic hearts pretreated with 0.1 μM Des-Arg^9-BK; Leu^8 + DesA^9: pretreated with Lys [Leu^8] Des-Arg^9-BK 1 μM before 0.1 μM Des-Arg^9-BK. Results are expressed as means ± s.e.mean (vertical bars) in pmol g^{-1} 5 min^{-1} of heart, $n = 5-8$. *$P<0.05$ compared with Isch; †$P<0.05$ compared with BK 0.1 μM or Des-Arg^9-BK; NS = not significant.

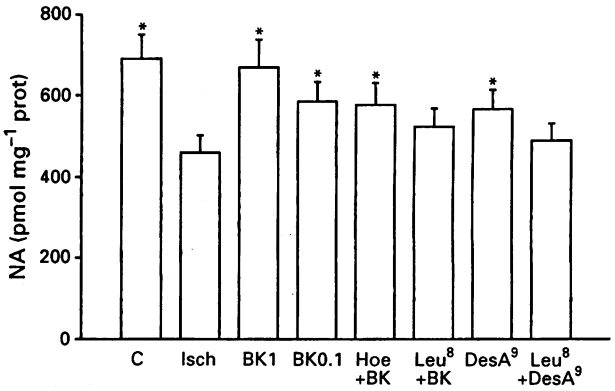

Figure 2 Myocardial noradrenaline (NA) content in isolated hearts submitted to 5 min reperfusion following 30 min stop flow. C: control hearts perfused during 35 min; Isch: ischaemic untreated hearts; BK 0.1: ischaemic hearts pretreated with 0.1 μM bradykinin; BK 1: pretreated with 1 μM bradykinin; Hoe + BK: ischaemic hearts pretreated with 1 μM Hoe 140 before 0.1 μM bradykinin; Leu^8 BK: pretreated with Lys [Leu^8] Des-Arg^9-BK 1 μM before 0.1 μM bradykinin; DesA^9: ischaemic hearts pretreated with 0.1 μM Des-Arg^9-BK; Leu^8 + DesA^9: pretreated with Lys [Leu^8] Des-Arg^9-BK 1 μM before 0.1 μM Des-Arg^9-BK. Results are expressed as means ± s.e.mean (vertical bars) in pmol mg^{-1} of heart protein content. $n = 5-8$. *$P<0.05$ compared with Isch.

Table 3 Concentrations of bradykinin (BK) measured in the Krebs-Henseleit (KH) buffer before the perfusion and in the perfusate buffer after their administration to the heart ($n = 5$)

BK concentration (μM) in KH buffer	*BK concentration (μM) in perfusate*	*Decrease (%)*
1	0.25 ± 0.03	− 75
0.1	0.05 ± 0.008	− 50
0.01	0.00375 ± 0.00095	− 62.5

Discussion

In 1973, Kimura *et al.* showed that during myocardial ischaemia induced by coronary ligation in anaesthetized dogs, bradykinin and bradykininase concentrations in coronary sinus blood were increased, while that of bradykininogen was decreased. In the dog model, levels of bradykinin varied considerably owing probably to differences in the development of collateral circulation and to the method of quantification. Recently, the beneficial effects of

bradykinin on myocardial ischaemia/reperfusion have been studied by various authors. Antiarrhythmic effects and improvement of cardiac performance after bradykinin infusion were observed in different animal species: pigs (Tio *et al.*, 1991), dogs (Linz & Schölkens, 1987) and rat isolated heart (Linz *et al.*, 1990). Carlsson & Abrahamsson (1989) have also reported that bradykinin (0.01–10 nM) reduced significantly noradrenaline efflux during reperfusion following total ischaemia in rat isolated hearts. Both of the latter studies used a constant rate of perfusion.

Catecholamines and their oxidation byproducts are known to have important deleterious effects accompanying reperfusion damages. In this context, Schömig *et al.* (1985) have shown that noradrenaline overflow during reperfusion represents a washout of the transmitter released from sympathetic nerve endings previously accumulated in the tissue during the ischaemic period. Noradrenaline is released via an inversion of the carrier system normally responsible for $uptake_1$ as a consequence of the metabolic imbalance that occurs within the nerve endings. It has also been shown that neuronal activity from a central origin does not lead to a substantial accumulation of noradrenaline within the ischaemic myocardium and that the intramyocardial noradrenaline stocks are expected to play the major role in modulating the transmitter release. Hence, the depletion of endogenous noradrenaline stores by α-methyl-*meta*-tyrosine reduces ventricular fibrillation and mortality in rats submitted to coronary artery ligation (Abrahamsson *et al.*, 1985).

It is important to mention that Rösen *et al.* (1983) have shown that in isolated perfused hearts bradykinin increased the rate of glucose uptake and oxidation as well as the formation of lactate, enhanced the nutritional flow across the capillary wall and indirectly glucose metabolism. Bradykinin caused also a concentration-dependent release of nitric oxide and of prostacyclin (Palmer *et al.*, 1987; Van Gilst *et al.*, 1987) which are known to block noradrenaline release and possess several pharmacological properties which render them potentially useful for the protection of the ischaemic myocardium. This could be an important contributing factor for myocardial tissue preservation.

In this study (using rat isolated hearts perfused at constant pressure) we have attempted to establish a link between arrhythmias and noradrenaline output at reperfusion. Because the amounts of noradrenaline released does not reflect those that are lost from the tissue (Lamontagne *et al.*, 1991), myocardial noradrenaline contents were also evaluated. Moreover, owing to the non-reproducibility of sampling in areas affected by coronary ligation (regional ischaemia), we used the model of acute total ischaemia to induce a global attack throughout the whole heart.

The results obtained showed that VF was prevented and VT significantly reduced only in hearts pretreated with bradykinin (1 μM). Under these conditions, noradrenaline release was completely abolished, while myocardial noradrenaline stocks were preserved. Thus bradykinin appears to reduce noradrenaline overflow after ischaemia and thereby affords a protection from arrhythmias.

It is conceivable that cells previously in anoxia react abnormally to a sudden oxygenation (Hess & Manson, 1984). Indeed, it has been shown that at reperfusion, there is enzyme leakage, increase of intracellular osmotic pressure, increased membrane permeability and an important Ca^{2+} entry resulting in an ionic alteration and a change of conduction velocity which, in the presence of catecholamines, may predispose the heart to arrhythmias.

Reperfusion-induced arrhythmias are resistant to most antiarrhythmic drugs (Naito *et al.*, 1981). Vasodilator drugs by decreasing cardiac afterload and by improving ventricular performance may prevent ventricular fibrillation following spasm or restoration of blood flow, a phenomenon that appears to be involved in sudden death.

One of the most important physiological effects of bradykinin is vasodilatation. However, as reported by Xiang *et al.* (1985), a great difference in cardiodynamics was observed between species when bradykinin was perfused in isolated hearts. Thus, bradykinin causes an increase of contractility in guinea-pigs, a decrease in rats and no change in rabbits. While coronary flow increased markedly in rabbits and guinea-pigs, the increase was moderate in rats. In the present study, the vasodilator effect of bradykinin was rather modest and not statistically significant: no change in heart rate was noted.

Bradykinin can be metabolized by various peptidases (Ward, 1991). Kininase I is well known to generate the active metabolite Des-Arg^9-BK, which has been shown to act via B_1 receptors. Using a specific carboxyterminal RIA for bradykinin quantification, we could not recover in the perfusate effluent the initial concentration added to the fluid perfusion. The low recovery of intact bradykinin pleads for an important metabolism of bradykinin by the myocardium. Indeed, Des-Arg^9-BK has been shown to be quite active in preventing ventricular fibrillation and noradrenaline overflow. This supports the hypothesis that B_1 receptors may be involved in the cardioprotective effect of bradykinin. This interpretation is supported by the results with antagonists. Thus, Hoe 140, the specific antagonist for B_2 receptor, which has been shown to antagonize the pharmacological effects of bradykinin in different *in vitro* models (Hock *et al.*, 1991) failed to demonstrate any significant effect on the bradykinin-mediated cardioprotection. On the contrary, Lys [Leu^8] Des-Arg^9-BK the specific antagonist of B_1 receptors abolished the effects of Des-Arg^9-BK and partially those of bradykinin. These observations, together with the low levels of bradykinin measured in the perfusate output versus those added initially to the perfusion medium suggest that the pharmacological effects of bradykinin may in part be due to its conversion to Des-Arg^9-BK and activation of B_1 receptors; this latter being expressed during the ischaemic period as suggested by Marceau & Regoli (1991) who noticed an induction of B_1 receptors in pathological conditions such as anoxia.

This work was supported by a grant from the Heart and Stroke Foundation of Québec.

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(Received May 28, 1992
Revised August 27, 1992
Accepted September 23, 1992)