Br. J. Pharmacol. (1988), **95**, 163–168

$GABA_A$-receptor-mediated inhibition of the delayed increase in intragastric pressure to stimulation of vagal afferent fibres in cats

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1 The possible involvement of γ-aminobutyric $acid_A$ ($GABA_A$)- and $GABA_B$-receptors in the inhibitory effects of GABA on the delayed increase in intragastric pressure of the stomach to stimulation of vagal afferent fibres in cats was studied.

2 Cats were anaesthetized with pentobarbitone-gallamine and pretreated with hexamethonium. GABA inhibited the hexamethonium-resistant delayed contraction of the stomach in a dose-dependent manner. Such effects of GABA were antagonized by both bicuculline and picrotoxin.

3 Muscimol, a $GABA_A$-receptor agonist, mimicked the inhibitory effects of GABA and the effects of muscimol were antagonized by bicuculline and picrotoxin. The ID_{50} of muscimol was 10 times less than that of GABA.

4 In contrast to muscimol, baclofen, a $GABA_B$-receptor agonist did not mimic the inhibitory effects of GABA.

5 The present experiments demonstrate that $GABA_A$-receptors are involved in the inhibitory action of GABA on the delayed contraction of the stomach to vagal afferent stimulation.

Introduction

Recently we demonstrated that electrical stimulation of the vagal trunk in pentobarbitone-gallamine anaesthetized cats produced a biphasic contractile response of the stomach (Kurahashi *et al.*, 1983; Okamoto *et al.*, 1986). The response was composed of an initial contraction to stimulation of the vagal efferent fibres during the stimulation period and a delayed contraction to stimulation of the vagal afferent fibres after the stimulation period. The initial contraction was sensitive to both hexamethonium and atropine, while the delayed contraction was sensitive to atropine but not to hexamethonium.

γ-Aminobutyric acid (GABA) has three effects on intestinal motility. In some experiments it has been found to stimulate the smooth muscle indirectly by exciting cholinergic neurones and, in areas of intestine where relaxations can be recorded easily, GABA stimulates enteric inhibitory neurones (Hobbiger, 1958; Inouye *et al.*, 1960; Lewis *et al.*, 1972; Krantis *et al.*, 1980). GABA also depresses the contractions of the small intestine caused by stimulation of enteric cholinergic neurones (Ong & Kerr, 1983). However, there are few accounts of the effect of GABA on stomach motility. Okamoto *et al.* (1986) showed that the delayed contraction of the stomach to stimulation of the vagal afferent fibres is inhibited by GABA. These inhibitory effects of GABA were antagonized by both picrotoxin and bicuculline. Thus, it was likely that the effects of GABA were mediated by the GABA-receptor-ionophore complex (Okamoto *et al.*, 1987).

However, GABA receptors are not homogeneous, there being at least two groups, $GABA_A$- and $GABA_B$-receptors (Hill & Bowery, 1981). The definition of GABA-receptor subtypes depends on selective responsiveness to various agonists and antagonists (Bowery *et al.*, 1981). The $GABA_A$-receptor is coupled to a Cl^--ionophore and is bicuculline- and picrotoxin-sensitive. The $GABA_B$-receptor is not associated with a Cl^--ionophore and is bicuculline-insensitive (Bowery *et al.*, 1981). While GABA itself is an agonist for both $GABA_A$- and $GABA_B$-receptors, muscimol is a specific $GABA_A$-receptor agonist and baclofen is a specific

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$GABA_B$-receptor agonist (Bowery *et al.*, 1981). Ong & Kerr (1983) found that both $GABA_A$- and $GABA_B$- receptors mediate the modification of intestinal motility by GABA in guinea-pigs. Whereas Bowery *et al.* (1979) demonstrated that $GABA_B$-receptors contribute to the inhibition of noradrenaline release from the atrium of rats and to the inhibition of contraction of the vas deferens in mice.

The present experiments were undertaken to investigate the involvement of $GABA_A$- and/or $GABA_B$-receptors in the inhibitory effects of GABA on the delayed contraction of the stomach to stimulation of the vagal afferent fibres in cats treated with hexamethonium.

Methods

The experimental procedures were essentially similar to those of Okamoto *et al.* (1986) and Tsubomura *et al.* (1987).

Twenty-five cats of either sex, weighing 2.5 to 4.0 kg, were used. The animals were deprived of food but allowed free access to water for 12 h before experiments. After initial anaesthesia with ether, pentobarbitone sodium (10 mg kg^{-1}, i.v.) was administered, additional amounts being administered when necessary. A tracheal cannula was inserted. The right femoral vein was catheterized and gallamine triethiodide (20 mg kg^{-1}) was continuously infused at a constant rate (1.48 ml h^{-1}). All animals were pretreated with hexamethonium (10 mg kg^{-1}). Artificial respiration was maintained by a respiration pump. The respiration rate was 15 min^{-1} with the air volume at 70 ml per stroke. The left femoral vein was catheterized for drug injection. The cervical vagal trunks on both sides were cut and the ends ligated. The distal trunk of the left vagus was placed on bipolar platinum electrodes and covered with cotton wool soaked in liquid paraffin. The cats were pretreated with propranolol (1 mg kg^{-1}) and phentolamine (2 mg kg^{-1}) to inhibit α- and β-adrenoceptors. Gastric motility was recorded with a balloon introduced via the oesophagus. The system was filled with water and connected to a pressure transducer; thus changes in intragastric pressure were recorded. The level of intragastric pressure was set at 10 to 15 cmH_2O and changes recorded on a polygraph (San-ei Instrument, Tokyo, Japan) via a pressure transducer. A stimulator giving square wave pulses was used (10 Hz in frequency, 3 ms in duration and 15 V in intensity for 10 s).

The drugs used were as follows: γ-aminobutyric acid (GABA), ether and hexamethonium (Nakarai Chemical Co., Kyoto, Japan); gallamine triethiodide, muscimol and bicuculline (Sigma Chemical Co., St. Louis, U.S.A.); picrotoxin (Wako Chemical Co., Osaka, Japan); baclofen (Ciba Geigy, Osaka, Japan).

The height of the contractile response of the stomach before administration of drugs was regarded as 100%.

Statistical analysis was performed by use of Student's *t* test for unpaired data.

Table 1 ID_{50} values of GABA, muscimol and baclofen on the hexamethonium-resistant increase in intragastric pressure to stimulation of the vagal trunk in cats

Agonist	ID_{50} ($\mu g\,kg^{-1}$)
GABA	43.0 ± 3.0
Muscimol	5.3 ± 2.7
Baclofen	> 1000

Results

In hexamethonium-treated cats, stimulation of the distal end of the ligated vagal trunk with electrical pulses (10 Hz, 15 V, 3 ms for 10 s) produced a delayed increase in intragastric pressure after the stimulation period, as described previously (Okamoto *et al.*, 1987).

Effects of GABA, muscimol and baclofen on the delayed contractile response

The effects of GABA, muscimol and baclofen on the delayed contraction of the stomach to stimulation of the vagal trunk were studied in 13 cats. Drugs were administered 1 min before stimulation of the vagus. The basal tone of the stomach was not affected by GABA, muscimol or baclofen. In 5 cats, the delayed contraction was significantly inhibited by the administration of GABA (10 to 100 $\mu g\,kg^{-1}$) in a dose-dependent manner (Figures 1a and 2a). Such inhibition did not continue for more than 15 min. In another 5 cats, muscimol (1 to 10 $\mu g\,kg^{-1}$) caused significant inhibition of the delayed contraction in a dose-dependent manner (Figures 1b and 2b). In the remaining 3 cats, baclofen (100 to 1000 $\mu g\,kg^{-1}$) did not significantly inhibit the response (Figures 1c and 2c). Judging from the ID_{50} values for inhibition of the delayed contraction, muscimol was 10 times more potent than GABA (Table 1).

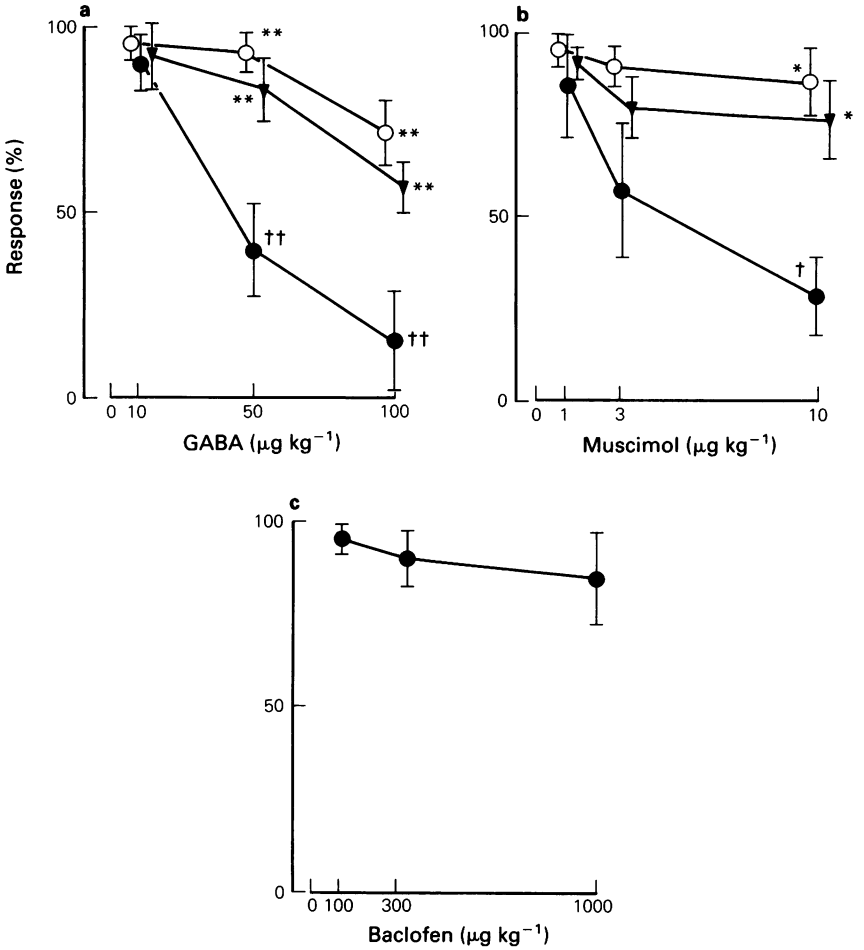

Figure 1 Effects of (a) GABA, (b) muscimol and (c) baclofen on the hexamethonium-resistant increase in intragastric pressure to stimulation of the vagal trunk in cats. (a) Effects of GABA (10 to 100 $\mu g\,kg^{-1}$). Ordinate scale; % response, as compared with control response before administration of GABA. Control (●), pretreatment with picrotoxin (500 $\mu g\,kg^{-1}$) (○), pretreatment with bicuculline (2 $mg\,kg^{-1}$) (▼). ††Indicates significant difference from corresponding control ($P < 0.01$). **Indicates significant difference from corresponding GABA-induced inhibition ($P < 0.025$, Student's t tests). (b) Effects of muscimol (1 to 10 $\mu g\,kg^{-1}$). Symbols as in (a). †Indicates significant difference from corresponding control ($P < 0.01$). *Indicates significant difference from corresponding muscimol-induced inhibition ($P < 0.01$, Student's t test). (c) Effects of baclofen (100 to 1000 $\mu g\,kg^{-1}$).

Effects of bicuculline and picrotoxin on GABA- or muscimol-induced inhibition of the delayed increase in intragastric pressure

A further set of experiments was conducted in 12 cats to determine the effects of bicuculline and picrotoxin on the inhibitory effects of GABA and muscimol on this gastric response to stimulation of the vagal trunk. Bicuculline (2 $mg\,kg^{-1}$) or picrotoxin (500 $\mu g\,kg^{-1}$) was administered to 3 cats 5 min before the administration of GABA. Neither bicuculline nor picrotoxin affected the basal tone of the stomach. The control response was recorded, then GABA (10 to 100 $\mu g\,kg^{-1}$) was administered 1 min before electrical stimulation. The pretreatment with bicuculline (data not shown) or picrotoxin antagonized the inhibitory effects of GABA (Figures 1a and 2a). The effects of bicuculline or picrotoxin continued for about 30 to 60 min. The effects of bicuculline (2 $mg\,kg^{-1}$) and picrotoxin (500 $\mu g\,kg^{-1}$) on the inhibitory actions of muscimol (1 to 10 $\mu g\,kg^{-1}$) were studied in another 6 cats, and it

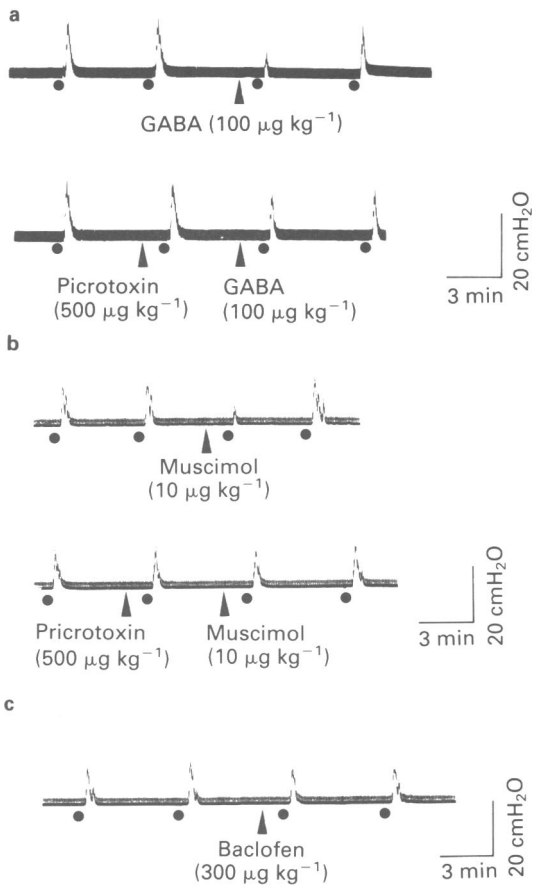

Figure 2 Representative recordings of the effects of (a) GABA, (b) muscimol and (c) baclofen on the hexamethonium-resistant increase in intragastric pressure to electrical stimulation of the vagal trunk in cats. Vertical scale indicates 20 cmH_2O, horizontal scale 3 min. Stimulation of the vagal trunk (●). (a) Effect of GABA (100 $\mu g\,kg^{-1}$) and antagonism by picrotoxin (500 $\mu g\,kg^{-1}$). (b) Effects of muscimol (10 $\mu g\,kg^{-1}$) and antagonism by picrotoxin (500 $\mu g\,kg^{-1}$). (c) Effects of baclofen (300 $\mu g\,kg^{-1}$).

was found that pretreatment with bicuculline or picrotoxin attenuated the inhibitory effects of muscimol significantly (Figures 1b and 2b). Since baclofen did not affect the delayed contraction, the effects of bicuculline or picrotoxins in combination with this drug were not studied.

Discussion

Both $GABA_A$- and $GABA_B$-receptors have been described in the guinea-pig intestine (Bowery *et al.*, 1979). GABA facilitates the release of acetylcholine and causes dose-dependent contractions in the guinea-pig ileum (Krantis & Kerr, 1981a; Kleinrok & Kilbinger, 1983). The response is of neural origin since the GABA-induced contraction is blocked by tetrodotoxin. The effects of GABA are antagonized by bicuculline and picrotoxin. Furthermore, muscimol mimics the effects of GABA. Thus, the excitatory effects of GABA in the guinea-pig ileum are mediated through bicuculline-sensitive $GABA_A$-receptors, coupled to a Cl^--ionophore (Ong & Kerr, 1983). Another action of GABA in the guinea-pig ileum is to depress electrically-evoked cholinergic contractions (Bowery *et al.*, 1979; Kleinrok & Kilbinger, 1983). This effect of GABA is not antagonized by either bicuculline or picrotoxin. Furthermore, muscimol does not mimic this effect of GABA while baclofen does. Thus, the inhibitory effects of GABA on the electrically-evoked cholinergic contraction are considered to be mediated through $GABA_A$- receptors (Ong & Kerr, 1983).

Since GABA dose-dependently inhibited the hexamethonium-resistant delayed increase in intragastric pressure and these effects of GABA were antagonized by bicuculline and picrotoxin, it is probable that this effect of GABA is mediated by the GABA-receptor-ionophore-complex (Okamoto *et al.*, 1987). In this study, low doses of muscimol mimicked the effect of GABA. Bicuculline and picrotoxin antagonized the effects of muscimol. In contrast to muscimol, baclofen had no effect on the delayed contraction. Thus, it seems likely that this inhibitory effect of GABA is mediated by bicuculline-sensitive $GABA_A$-receptors.

GABA reduces the release of acetylcholine from preganglionic nerve terminals of frog sympathetic ganglia, an effect not blocked by picrotoxin (Kato & Kuba, 1980). Kleinrok & Kilbinger (1983) demonstrated that the depressant effects of GABA on electrically-evoked acetylcholine release and contraction in guinea-pig ileum are mediated through bicuculline- and picrotoxin-insensitive GABA receptors. They suggested that these GABA-receptors are localized presynaptically as in frog sympathetic ganglia. Bicuculline-sensitive $GABA_A$-receptors mediate the postsynaptic inhibitory effects of GABA in the cat spinal cord (Curtis *et al.*, 1971). In the mouse spinal cord, barbiturates enhance bicuculline-sensitive $GABA_A$-mediated postsynaptic inhibition (Schulz & Macdonald, 1981; Olsen, 1982). Recently, we found that pentobarbitone enhanced the inhibitory effects of GABA on the delayed contractile response of the stomach (Okamoto *et al.*, 1987). Thus, it is possible that this $GABA_A$-receptor is localized postsynaptically. However, both pre- and post-synaptic locations of bicuculline-sensitive $GABA_A$-receptors have been described (Curtis &

Johnston, 1974; De Feudis, 1977). Further experiments are needed to elucidate whether GABA acts presynaptically or postsynaptically in the preparation used in the present study.

There are two types of inhibitory nerves in the guinea-pig intestine: noradrenergic (Furness & Costa, 1974) and intrinsic enteric inhibitory nerves (Furness & Costa, 1973). In this preparation, GABA stimulates intrinsic enteric inhibitory nerves but not terminals of inhibitory noradrenergic nerves. In this study, cats were pretreated with α- and β-adrenoceptor antagonists. Thus, it is unlikely that adrenergic nerves contribute to the inhibitory effects of GABA on the delayed contractile response. In the guinea-pig intestine, $GABA_A$-receptor antagonism by bicuculline or by tachyphylaxis to GABA slows peristalsis and often reduces the amplitude of the reflex contraction. This supports the view that the $GABA_A$-receptor may contribute to the propulsive activity of the guinea-pig distal colon (Krantis & Kerr, 1981b). Delbro *et al.* (1982) suggested that the hexamethonium-resistant gastric motor response to antidromic activation of vagal afferent fibres may involve vagal axon reflexes in cats. Our results indicate that $GABA_A$-receptors may modulate these vagal axon reflexes.

This study was supported in part by a Grant-in-Aid from the Smoking Research Foundation, Japan.

References

BOWERY, N.G., DOBLE, A., HILL, D.R., HUDSON, A.L., SHAW, J.S. & TURNBULL, M.J. (1979). Baclofen: a selective agonist for a novel type of GABA receptor. *Br. J. Pharmacol.*, **67**, 444–445.

BOWERY, N.G., DOBLE, A., HILL, D.R., HUDSON, A.L., SHAW, J.S., TURNBULL, M.J. & WARRINGTON, R. (1981). Bicuculline-insensitive GABA receptors on peripheral autonomic nerve terminals. *Eur. J. Pharmacol.*, **71**, 53–70.

CURTIS, D.R., DUGGAN, A.W., FELIX, D. & JOHNSTON, G.A.R. (1971). Bicuculline, an antagonist of GABA and synaptic inhibition in the spinal cord of the cat. *Brain Res.*, **32**, 69–96.

CURTIS, D.R. & JOHNSTON, G.A.R. (1974). Amino-acid transmitters in the mammalian central nervous system. *Ergebn. Physiol.*, **69**, 97–188.

DE FEUDIS, F.V. (1977). GABA-receptors in the vertebrate nervous system. *Prog. Neurobiol.*, **9**, 123–145.

DELBRO, D., FÄNDRIKS, L., LISANDER, B. & ANDERSSON, S.A. (1982). Gastric atropine-sensitive excitation by peripheral vagal stimulation after hexamethonium. Antidromic activation of afferents? *Acta Physiol. Scand.*, **114**, 433–440.

FURNESS, J.B. & COSTA, M. (1973). The nervous release and the action of substances which affect intestinal muscle through neither adrenoreceptors nor cholinoreceptors. *Phil. Trans. Roy. Soc. B.*, **265**, 123–133.

FURNESS, J.B. & COSTA, M. (1974). The adrenergic innervation of the gastrointestinal tract. *Ergebn. Physiol.*, **69**, 1–57.

HILL, D.R. & BOWERY, N.G. (1981). (^{3}H)-Baclofen and (^{3}H)-GABA binding to bicuculline-insensitive $GABA_B$ sites in rat brain. *Nature*, **290**, 149–152.

HOBBIGER, F. (1958). Effects of γ-aminobutyric acid on the isolated mammalian ileum. *J. Physiol.*, **142**, 147–164.

INOUYE, A., FUKUYA, M., TSUCHIYA, K. & TSUJIOKA, T. (1960). Studies on the effects of γ-aminobutyric acid on the isolated guinea-pig ileum. *Jpn. J. Physiol.*, **10**, 167–182.

KATO, E. & KUBA, K. (1980). Inhibition of transmitter release in bullfrog sympathetic ganglia induced by γ-aminobutyric acid. *J. Physiol.*, **298**, 271–283.

KLEINROK, A. & KILBINGER, H. (1983). γ-Aminobutyric acid and cholinergic transmission in the guinea-pig ileum. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **322**, 216–220.

KRANTIS, A., COSTA, M., FURNESS, J.B. & ORBACH, J. (1980). γ-Aminobutyric acid stimulates intrinsic inhibitory and excitatory nerves in the guinea-pig intestine. *Eur. J. Pharmacol.*, **67**, 461–468.

KRANTIS, A. & KERR, D.I.B. (1981a). GABA-induced excitatory response in the guinea-pig small intestine are antagonized by bicuculline, picrotoxin and chloride ion blockers. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **317**, 257–261.

KRANTIS, A. & KERR, D.I.B. (1981b). The effect of GABA antagonism on propulsive activity of the guinea-pig large intestine. *Eur. J. Pharmacol.*, **76**, 111–114.

KURAHASHI, K., FUJIWARA, M. & TANIGUCHI, T. (1983). Cholinergic excitatory response of stomach to stimulation of the vagal trunk in cats with chronic supranodose vagotomy. *IRCS Med. Sci.*, **11**, 62.

LEWIS, G.P., McMARTIN, C., ROSENTHAL, S.R. & YATES, C. (1972). Isolation and identification of pharmacologically active amino acids in skin and their structure-activity relationship on the guinea-pig ileum. *Br. J. Pharmacol.*, **45**, 104–117.

OKAMOTO, T., KURAHASHI, K., TSUBOMURA, T. & FUJIWARA, M. (1986). Effects of morphine on hexamethonium-sensitive and -resistant excitatory responses of stomach to stimulation of vagal trunk in cats. *Life Sci.*, **39**, 147–153.

OKAMOTO, T., KURAHASHI, K., TSUBOMURA, T. & FUJIWARA, M. (1987). Effect of barbiturates on the inhibitory action of GABA on excitatory response of the stomach to stimulation of vagal afferent fibres in cat. *Life Sci.*, **41**, 405–411.

OLSEN, R.W. (1982). Drug interactions at the GABA receptor-ionophore complex. *Ann. Rev. Pharmacol. Toxicol.*, **22**, 245–277.

ONG, J. & KERR, D.I.B. (1983). $GABA_A$- and $GABA_B$-receptor mediated modification of intestinal motility. *Eur. J. Pharmacol.*, **86**, 9–17.

SCHULZ, D.W. & MACDONALD, R.L. (1981). Barbiturate

enhancement of GABA-mediated inhibition and activation of chloride ion conductance: correlation with anticonvulsant and anesthetic actions. *Brain Res.*, **209,** 177–188.

TSUBOMURA, T., OKAMOTO, T., KURAHASHI, K. & FUJIWARA, M. (1987). Gastric excitation by stimulation of the vagal trunk after chronic supranodose vagotomy in cats. *J. Pharmacol. Exp. Ther.*, **241,** 650–654.

(Received February 9, 1988
Revised April 12, 1988
Accepted April 16, 1988)