Br. J. Pharmac. (1983), **80**, 691–697

Heterogeneity of 5-hydroxytryptamine receptors in the rat uterus and stomach strip

Susan J. Wrigglesworth[1]

Department of Pharmacology, University College London, Gower Street, London WC1

1 Experiments were performed using the rat isolated uterus and the rat stomach strip to investigate the effects of 5-hydroxytryptamine (5-HT) on 5-HT receptors in the presence of different antagonists. Amitriptyline, methysergide and trazodone were used to antagonize the response to 5-HT in these tissues.

2 The pA_2 value for amitriptyline with 5-HT as the agonist was estimated from the Schild plot analysis and found to differ significantly ($P<0.05$) in the stomach strip (6.36) from that in the isolated uterus (9.06).

3 A similar difference was found using trazodone; here the pA_2 value was 6.74 in the stomach strip and 8.49 in the isolated uterus.

4 These results indicate a difference between the two tissues. This difference is discussed in terms of heterogeneity of 5-HT receptors.

Introduction

In 1957 the receptors for 5-hydroxytryptamine (5-HT) in smooth muscle were classified into two types: D- receptors which are blocked by phenoxybenzamine and M-receptors which act via release of acetylcholine and can be blocked by morphine (Gaddum & Picarelli, 1957; Brownlee & Johnson, 1963). Evidence has subsequently been put forward (Apperley, Feniuk, Humphrey & Levy, 1980) for two types of excitatory 5-HT receptors in the dog vasculature; one type similar to the classical D-receptor and the other with neither D-nor M-receptor characteristics. The existence of heterogeneous 5-HT receptors has also been indicated in the rat stomach strip (Frankhuijzen & Bonta, 1974 a,b; Glennon, Liebowitz & Mack, 1978), and in recent agonist-antagonist studies using the 5-HT antagonist ketanserin which has anti-hypertensive properties (Millar, Facoory & Laverty, 1982; van Nueten, Leysen & Schuurkes, 1983). Since there are no M-receptors in the rat stomach (Offermeier & Ariëns, 1966) or in the rat uterus (von Fanchamps, Doepfner, Weidemann & Cerletti, 1960) these tissues were used to study some of the properties of the D-type receptors for 5-HT in mammalian smooth muscle.

[1]Present Address: Charing Cross Hospital Medical School (University of London), The Reynolds Building, St. Dunstan's Road, London W6 8RP.

Methods

Agonist-antagonist interactions were used to compare the 5-HT receptor responses in the two tissues. The pA_2 values (Schild, 1957) were found for trazodone, a psychoactive drug known to have a potent anti-5-hydroxytryptaminergic action (Silvestrini, Cioli, Burberi & Catanese, 1968; Domino, 1973), and for amitriptyline which blocks noradrenaline and 5-HT uptake in the brain (Carlsson, Corroli, Fuke & Hökfelt, 1969). A further comparison of the responses to 5-HT in the stomach and uterus was made using methysergide.

Rat isolated fundic strip

The stomach strips were set up according to the method described by Vane (1957). Male hooded rats (150–300 g) were killed by a blow on the head and the stomach was dissected free from the abdomen. The fundic region of the stomach was cut away from the pyloric region and the fundal contents removed. The fundus was subsequently cut into two longitudinal strips parallel to the greater curvature; this preserved the longitudinal musculature. These strips were then suspended in 5 ml organ baths containing Tyrode solution of the following composition (mM): NaCl 136, KCl 2.7, NaH_2PO_4 0.42, $CaCl_2$ 1.8, $MgCl_2$ 1.6, $NaHCO_3$ 11.9, glucose 5.6, equilibrated with a slow stream of 95% O_2 and 5% CO_2. The temperature of this solution in the organ bath was kept

constant at 37°C by means of a regulated heating jacket. Four stomach strips were mounted in parallel organ baths and cumulative concentration-response curves were registered using an isotonic transducer connected to a pen recorder. After two control concentration-response curves had been completed with 30 min rest between them, the tissues were allowed to return to basal length prior to the next concentration-response curve in the presence of the antagonist. This eliminated the effects of desensitization. A separate tissue was used for each antagonist concentration-response curve and the antagonist was left in contact with the tissue for 60 min before the third agonist concentration-response curve was made. This contact time was used for all tissues to avoid time-dependent changes.

Rat isolated uterus

Female hooded rats (150–200 g) in natural oestrus were killed by a blow on the head and the uterine horns were excised and separated from the fat deposits and ovaries. The middle 2 cm of the uterine horns were cut longitudinally in an attempt to facilitate access of the drugs to the tissue receptors. Each horn was mounted in a 5 ml organ bath containing low calcium Tyrode solution kept at 31°C and gassed with 95% O_2 and 5% CO_2. The composition of this solution was identical to that used for the stomach except that the $CaCl_2$ concentration was reduced to 1.0 mM. Drugs added to the baths were left in contact with the tissue for 40 s using a 6 min cycle. The baths were emptied and filled automatically (Boura, Mongar & Schild, 1954). Two control concentration-response curves to 5-HT were completed and then repeated in the presence of the antagonist as described for the stomach strip. The two control curves were used to detect a tissue with random variability; where the two controls were not identical the tissue was eliminated.

Statistical analysis

The EC_{50} values were estimated from the concentration-response curves; in the presence of an antagonist these were displaced to the right without depression of the maximum response, so the dose-ratios (x) could be estimated (van Rossum, 1963). The dose-ratio is the ratio of concentrations of the agonist in the presence and absence of antagonist which produce a 50% maximal response. Schild plots were constructed (Schild, 1947) for the antagonist-agonist interaction; the values of log $(x-1)$ were plotted against log I (antagonist concentration). Least squares linear regression analysis of the Schild plots was used to obtain the pA_2 value for the competitive antagonism by extrapolation of the line to zero. Confidence limits ($P=0.05$) were calculated for the pA_2 value and the slope of the regression line. In each regression analysis there were four points at each antagonist concentration. Probabilities were calculated using Student's t test and P values less than 0.05 were considered to be significant.

Drugs

Amitriptyline hydrochloride (Roche), atropine sulphate (BDH), bethanechol (Sigma), 5-hydroxytryptamine creatinine sulphate (Sigma), 5-methoxytryptamine hydrochloride (Sigma), mepyramine maleate (May & Baker), methysergide hydrogenmaleate (Sandoz) and trazodone hydrochloride (Roussel) were used.

Results

The antagonist action of trazodone

Rat stomach strip. Trazodone (10^{-7}–10^{-4} M) antagonized the isolated stomach strip response to 5-HT, causing displacement of the 5-HT concentration-response curves to the right of the control (Figure 1). The dose-response obtained from these experiments gave a Schild plot with a regression slope of 1.19 (95% confidence limits 1.02–1.31) and a pA_2 estimate of 6.74 (6.5–7.0) (Figure 2 and Table 1). After equilibration of the stomach strip for 1 h with high concentrations of trazodone (3.0×10^{-5} M and 10^{-4} M) relaxation equivalent to 15% of the maximal response to 5-HT was observed. A correction was made for this relaxation on the graph by taking the new resting value as the base. Washing out the trazodone from the tissue bath for 1 h reversed

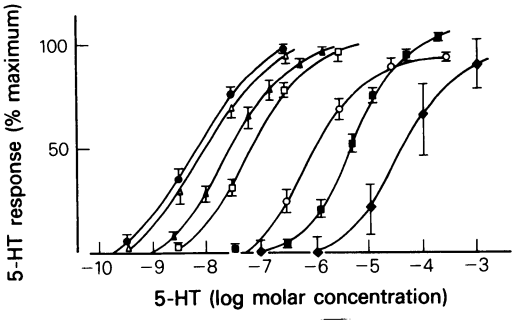

Figure 1 The effect of trazodone on the rat isolated stomach strip response to 5-HT. Control responses to 5-HT (●); responses to 5-HT in the presence of trazodone: 10^{-7} M, (△); 3×10^{-7} M, (▲); 10^{-6} M, (□); 10^{-5} M, (○); 3×10^{-5} M, (■); and 10^{-4} M, (◆). Vertical lines represent s.e.mean; $n = 24$ for the control and $n = 4$ for other curves.

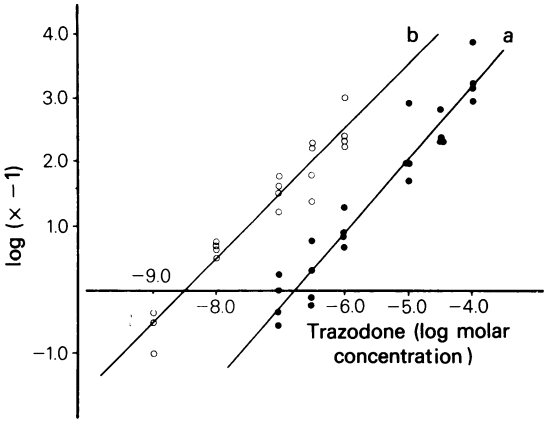

Figure 2 (●) Schild plot for trazodone antagonism of the response of the rat isolated stomach strip to 5-HT; pA_2 = 6.74 (6.5–7.0), slope = 1.19 (1.02–1.31). (○) Schild plot for trazodone antagonism of the rat isolated uterus response to 5-HT; pA_2 = 8.49 (8.28–8.78), slope = 0.99 (0.85–1.15). x = dose-ratio.

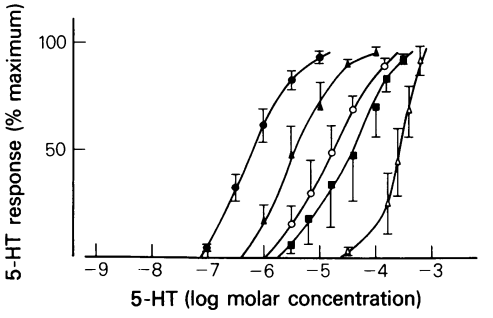

Figure 3 The effect of trazodone on the rat isolated uterus response to 5-HT. Control responses to 5-HT, (●); responses to 5-HT in the presence of trazodone: 10^{-8} M, (▲); 10^{-7} M, (○); 3×10^{-7} M, (■); and 10^{-6} M, (△). Trazodone 10^{-9} M produced a very small shift to the right of the control responses. Vertical lines represent s.e.mean; $n = 20$ for the control and $n = 4$ for other curves.

the antagonist effect. Further investigation of the competitive action of trazodone was made using the tryptamine analogue 5-methoxytryptamine. This analogue acts as a 5-HT receptor agonist but is less potent than 5-HT in its action (Vane, 1959). Trazodone was also found to antagonize the response of the stomach strip to 5-methoxytryptamine by causing a parallel displacement of the concentration-response curves without significant depression of the maxima. The pA_2 value estimated for trazodone against 5-methoxytryptamine was 6.65 which was not significantly different from that obtained with 5-HT. The slope of the Schild plot for 5-HT antagonized by 5-methoxytryptamine was significantly greater than unity (1.50) (Table 1).

Rat isolated uterus. The steep concentration-response curves for 5-HT on the uterus were also displaced to the right by trazodone (10^{-8}–10^{-6} M) without depression of the maxima (Figure 3). The concentrations of trazodone required for antagonism in the uterus were lower than those for the stomach and consequently the pA_2 value estimated in the uterus was significantly higher than that for the stomach 8.49, (8.28–8.78) (Table 1). The slope of the Schild plot for trazodone and 5-HT (Figure 2) was not significantly different from unity 0.99 (0.85–1.15). With the agonist 5-methoxytryptamine the pA_2 value for trazodone 7.91 (7.69–8.21) was similar to that for 5-HT and the regression slope for the Schild plot was 1.12 (0.90–1.33).

The antagonist action of amitriptyline

Rat stomach strip. Amitriptyline (10^{-6}–10^{-4} M) caused the concentration-response curves for 5-HT in the stomach strip to be displaced to the right (Figure 4). Amitriptyline caused the stomach strip to

Table 1 Schild plot analysis for trazodone and amitriptyline antagonism of rat isolated tissue responses to 5-HT and 5-methoxytryptamine (5-MOT)

	Uterus				*Stomach strip*			
Agonist	pA_2	*95% CL*	*Slope*	*95% CL*	pA_2	*95% CL*	*Slope*	*95% CL*
Trazodone vs 5-HT	8.49	8.28 8.78	0.99	0.85 1.15	6.74	6.50 7.00	1.19	1.02 1.31
Trazodone vs 5-MOT	7.91	7.69 8.21	1.12	0.90 1.33	6.65	6.40 7.00	1.50	1.21 1.78
Amitriptyline vs 5-HT	9.06	7.46 11.09	0.74	0.48 1.00	6.36	5.79 7.02	1.18	0.83 1.50

A fresh uterus or stomach strip was used for each experiment.

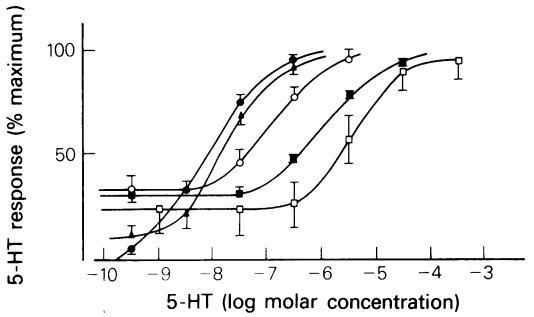

Figure 4 The effect of amitriptyline on the rat isolated stomach strip response to 5-HT. Control responses to 5-HT, (●); responses to 5-HT in the presence of amitriptyline: 10^{-6} M (▲); 10^{-5} M, (○); 3×10^{-5} M, (■); and 10^{-4} M, (□). Vertical lines represent s.e.mean; $n = 16$ for control and $n = 4$ for other curves.

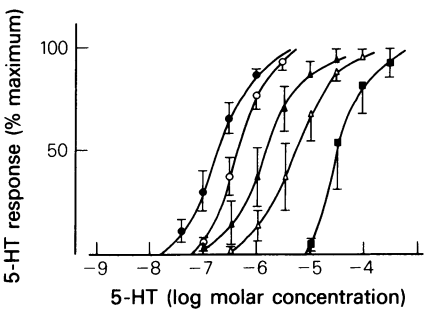

Figure 6 The effect of amitriptyline on the rat isolated uterus response to 5-HT. Control responses to 5-HT, (●); response to 5-HT in the presence of amitriptyline: 10^{-9} M, (○); 10^{-8} M, (▲); 10^{-7} M, (△) and 10^{-6} M, (■). Vertical lines represent s.e.mean; $n = 16$ for control and $n = 4$ for other curves.

contract during the 1 h equilibration period. This contraction was maintained and increased with higher concentrations of antagonist to an average maximum level which was 34% of that to 5-HT. The pA_2 value 6.36 (5.79–7.02) estimated from linear regression in the Schild plot (Figure 5) was not significantly different from the pA_2 values obtained using trazodone (Table 1). The slope of this Schild plot 1.18 (0.83–1.50) was not significantly different from unity thereby indicating competitive antagonism.

However, it was necessary to determine whether or not the pA_2 value estimated for amitriptyline was altered by its agonist action, and to investigate the mechanism of the contraction of the stomach strip. Methysergide (10^{-6} M) a potent 5-HT antagonist (Frankhuijzen & Bonta, 1974a) had no effect on the concentration-response curve to amitriptyline (10^{-6}–10^{-3} M), and therefore excluded the involvement of 5-HT receptors. The agonist action of amitriptyline was unaffected by both atropine (10^{-6} M) and idomethacin (10^{-5} M), indicating that an action on muscarinic receptors or the release of prostaglandins were not involved.

Concentration-response curves to bethanechol were obtained using the stomach strip and were found to be displaced to the right by amitriptyline, which again contracted the stomach during the equilibration period. The pA_2 value estimated for bethanechol and amitriptyline was 7.55 (7.34–7.82) with a Schild plot slope of 1.08 (0.91–1.24). Estimates of the pA_2 value for the same agonist-antagonist combination in the guinea-pig isolated ileum and trachea agree with the values found in the stomach strip (Angus & Gerskowitch, personal communication).

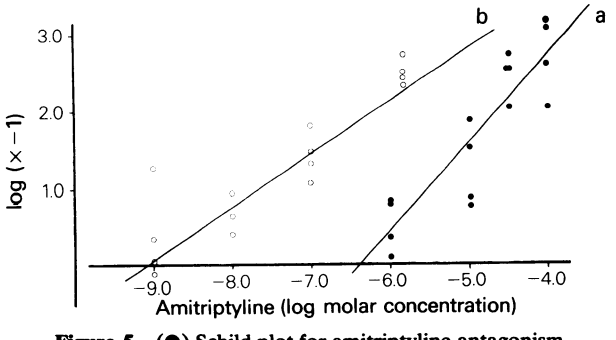

Figure 5 (●) Schild plot for amitriptyline antagonism of the rat stomach strip response to 5-HT; $pA_2 = 6.36$ (5.79–7.02), slope = 1.18 (0.81–1.50). (○) Schild plot for amitriptyline antagonism of the rat isolated uterus response to 5-HT; $pA_2 = 9.06$ (7.46–11.09), slope = 0.74 (0.48–1.00). x = dose ratio.

Rat isolated uterus. The rat uterus responses to 5-HT were antagonized by amitriptyline (10^{-9}–10^{-6} M), which displaced the concentration-response curves to the right (Figure 6). The antagonism was reversed after washing out the antagonist for 1 h. Amitriptyline had no observable agonist effect on the uterus when added to the tissue. The pA_2 value obtained was 9.06 (7.46–11.09) (Figure 5), which was significantly different from that of amitriptyline with 5-HT as the agonist in the stomach (Table 1). The slope of the Schild plot for the amitriptyline-5-HT interaction was not significantly different from unity 0.74 (0.48–1.00). A competitive antagonistic action was indicated for amitriptyline.

The bethanechol-amitriptyline interaction was investigated in the rat uterus so that a comparison could be made with that in the rat stomach. In the experiment on the uterus with bethanechol as the agonist the pA_2 value for amitriptyline was 7.53 (7.27–7.84) and the slope of the Schild plot regression line was 1.06 (0.87–1.25); these values were not significantly different from those in the stomach.

The antagonist action of methysergide

Rat stomach strip. The effects of 5-HT on the stomach strip were very sensitive to low concentrations of methysergide (10^{-10}–10^{-8} M) which depressed the maximum response to 5-HT. Increasing the concentration of methysergide increased the depression, displaced the concentration-response curves to the right and decreased their slopes ($P<0.05$); at 10^{-7} M methysergide completely abolished the response to 5-HT. Methysergide (8×10^{-10} M) decreased the maximum response to 5-HT by 50%. Such characteristics are typical of non-competitive antagonists. The antagonism was slowly reversed by washing the methysergide out of the tissue bath for 2 h.

Rat isolated uterus. Concentration-response curves to 5-HT in the uterus were displaced to the right by methysergide (3.0×10^{-11}–10^{-9} M). In addition, the maximum response to 5-HT was depressed and the slopes of the curves decreased ($P<0.05$). These characteristics, typical of non-competitive antagonism, were similar to those found in the stomach strip. Methysergide (10^{-9} M) abolished the response to 5-HT in the uterus and concentrations as low as 10^{-11} M were able to depress the maximum response. A 50% reduction of the maximum response was produced by 2×10^{-10} M methysergide. The antagonism was slowly reversed by washing the tissue for 2 h with Tyrode solution.

Discussion

The results confirm that 5-HT is a potent agonist causing contraction in both the rat uterus and stomach strip, but suggest that different types of 5-HT receptor may be involved in the responses of these two tissues. Trazodone and amitriptyline were more potent antagonists of 5-HT in the rat uterus than in the stomach strip. Trazodone acted as a competitive antagonist to both 5-HT and 5-methoxytryptamine (5-MOT) in the uterus, but there was a departure from the necessary conditions for competition when the stomach strip was used. Unlike 5-HT, it is known that 5-MOT enters the tissue cells (Vane, 1959; Maxwell, Gray & Taylor, 1961). If this entry were blocked by trazodone the pA_2 value for trazodone against 5-MOT would decrease and the slope of the Schild plot would increase. This could account for the lower pA_2 values for the 5-MOT-trazodone interaction and the higher slopes of the Schild plots in the uterus and the stomach.

The differences found here between the uterus and stomach strip could be indicative of different 5-HT receptors, but verification of this requires elimination of alternative explanations.

Detailed analysis of these results requires information on the mode of action of 5-HT and how the stages of the stimulus-response pathway are affected by trazodone. A delicate control of intracellular cyclic nucleotides and calcium ions is known to be important in many receptor responses (Amer & Kreighbaum, 1975). A difference between the forms of cyclic guanosine monophosphate phosphodiesterase present in the uterus and the stomach could possibly account for different sensitivities to trazodone. Alternatively, over or under estimation of the dose-ratios may occur if uptake processes for 5-HT in the two tissues differ or if a diffusion barrier limits the access of trazodone to the stomach muscle cells. Heterogeneous receptor subsites may also explain the different sensitivities to trazodone (Delean, Munson & Rodbard, 1978).

However, a similar difference between the pA_2 values for amitriptyline on the uterus and stomach was found. Amitriptyline showed competitive antagonism against the 5-HT response in both tissues, although an agonist action was observed with amitriptyline in the stomach strip. This agonist effect of amitriptyline may have altered its antagonist action towards the 5-HT response and consequently affected the pA_2 value in the stomach strip. Hence, the pA_2 values estimated for amitriptyline and other agonists would also have been affected. Amitriptyline is known to antagonize muscarinic receptors (Vernier, Hanson & Stone, 1962) and assuming that these receptors are identical in both the rat uterus and stomach the pA_2 values estimated for amitriptyline with a selective muscarinic receptor agonist such as bethanechol (Burleigh, 1978) should be identical in the two tissues. Before concluding that the 5-HT receptors in the uterus and stomach are different, the bethanechol-amitriptyline interaction was investigated in the rat uterus and stomach strip. Any difference in the pA_2 values obtained for the two tissues would imply that the agonist component of amitriptyline's action altered its activity as an antagonist. The results show that the pA_2 value found for the uterus was not significantly different from that in the stomach. The pA_2 values for the bethanechol-amitriptyline interaction in the uterus and stomach were similar to those found in the guinea-pig trachea (7.18) and ileum (7.45) given by Angus & Gerskowitch (unpublished results). The partial agonist action of amitriptyline in the stomach therefore does not alter the antagonist effect and the pA_2 estimation for bethanechol is valid. Assuming the contraction mechanisms triggered by stimulation of bethanechol and 5-HT receptors are the same, it is likely that the pA_2 values for the 5-HT-amitriptyline interaction are not altered either.

The Schild plot for the amitriptyline -5-HT interaction in the stomach could be analysed using the results at the three lowest concentrations and the three highest concentrations. Analysis using the low concentrations gives a decreased Schild plot slope and an increased pA_2 value. The inverse is true for the higher concentrations. This may be attributed to a heterogeneous receptor population sensitive to low concentrations of amitriptyline. If the results at low amitriptyline concentrations are excluded there is an increase in the Schild plot regression slope and a smaller pA_2 value, but the pA_2 value for 5-HT in the stomach is still greater than that in the uterus.

Methysergide, which is a well known antagonist of 5-HT (Offermeier & Ariëns, 1966) was found to have the characteristics of a non-competitive antagonist in the uterus and stomach strip. Interpretation of non-competitive antagonistic effects is indirect in terms of 5-HT receptors. However, methysergide was found to be a specific antagonist for 5-HT responses by von Fanchamps *et al.*, (1960) who found that methysergide does not antagonize responses to acetylcholine and has little or no effect on noradrenaline responses in peripheral tissues. So, assuming that methysergide is acting at a stage in the stimulus-response pathway peculiar to 5-HT or at a particular receptor site interconnected with the 5-HT receptor, the results indicate that the uterus response to 5-HT is more sensitive to methysergide than that of the stomach.

Recent work using the 5-HT antagonist ketanserin which has anti-hypertensive properties has also shown heterogeneity between the 5-HT receptors in the rat uterus (Millar, Facoory & Laverty, 1982) and rat stomach strip (van Nueten *et al.*, 1983). Ketanserin antagonizes the response to 5-HT in the rat uterus and rat caudal artery but not in the rat stomach strip. Characterization of the heterogeneous 5-HT receptor population in peripheral tissues is essential if antihypertensive drugs such as ketanserin, which inhibit the vascular contractile response to 5-HT, are to be used in clinical situations.

In conclusion the results presented here show differences between the receptors for 5-HT in the rat stomach and those in the uterus. This is consistent with the existing evidence for heterogeneous 5-HT receptors in peripheral tissues and shows that the 5-HT receptors in the stomach are more sensitive to certain 5-HT antagonists than those in the uterus.

This work was supported by a grant from the Medical Research Council of Great Britain. I wish to thank Dr R. Rips and Dr Gerskowitch for their encouragement and help in the preparation of this article.

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(*Received April 12, 1983.*
Revised July 18, 1983.)