British Journal of Pharmacology (1996) 119, 519-526

The effect of 5-HT and selective 5-HT receptor agonists and antagonists on rat dorsal vagal preganglionic neurones *in vitro*

[1]Anthony P. Albert, K. Michael Spyer & Penelope A. Brooks

Department of Physiology, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF

1 Whole-cell patch-clamp recordings were made from 142 visually identified rat dorsal vagal preganglionic neurones (DVMs). Applications of 5-hydroxytryptamine (5-HT, 20 μM, 2 min) elicited a slow depolarization (8.2 ± 0.5 mV, $n=59$) in 95% of the cells tested, accompanied by an increase in excitability. In (68%) of DVMs the depolarization was associated with an increase in apparent membrane resistance (R_m, 22.7 ± 2.2%). These depolarizations and increases in R_m (14.3 ± 2.6%, $n=8$) were maintained in a medium which blocked synaptic transmission.

2 The response to 5-HT was associated with a reversal potential (E_{rev}) of -91 ± 1 mV at an extracellular K^+ concentration ($[K^+]_o$) of 4.2 mM. This correlated well with the K^+ equilibrium potential ($E_K = -89$ mV).

3 The depolarizing effect of 5-HT was attenuated by the 5-$HT_{2A/2C}$ receptor antagonists, ketanserin (1 μM), LY 53,857 (1 μM) and the 5-$HT_{1A/2A}$ receptor antagonist, spiperone (1 μM). The 5-HT_{1A} receptor antagonist, pindobind 5-HT_{1A} (5 μM), had no effect on the depolarizing response to 5-HT.

4 The effect of 5-HT was mimicked by the 5-$HT_{2A/2C}$ receptor agonist, α-methyl-5-HT (50 μM), the 5-HT_1 receptor agonist, 5-carboxamidotryptamine (20 μM) and the putative 5-HT_4 agonist, 5-methyoxytryptamine (50 μM). The selective 5-HT_4 receptor antagonist, GR113808, had no effect on the depolarizing effect of 5-HT or 5-MEOT on DVMs.

5 The 5-HT_3 antagonists, MDL 72222 (10 μM) and ICS-205-930 (1 and 10 μM), partially reduced the effect of 5-HT. The 5-HT_3 receptor agonist, 2-methyl-5-HT (100-300 μM), excited a proportion of neurones tested (56%) by evoking a depolarizing and/or an increase in postsynaptic potentials (p.s.ps).

6 These results are consistent with direct, postsynaptic actions of 5-HT on DVMs via 5-HT_{2A} receptors, being mediated, in part, by the reduction of K^+ conductance.

Keywords: Whole-cell patch-clamp recording; dorsal vagal preganglionic neurones; 5-HT; potassium channels; 5-HT_{2A} receptor subtype

Introduction

The dorsal motor nucleus of the vagus (DMN) is located in the dorsomedial medulla oblongata. It mainly contains dorsal vagal preganglionic neurones (DVMs) (80%) which control many autonomic functions including gastric motility, gastric and pancreatic secretions and heart rate (Loewy & Spyer, 1992).

Immunocytochemical studies have shown that 5-hydroxytryptamine (5-HT) is present in nerve fibres and terminals innervating the DMN (Steinbusch, 1981). Electrophysiological and neuroanatomical evidence suggests that this 5-hydroxytryptaminergic input originates from raphe nuclei (Rogers *et al.*, 1980; Thor & Helke, 1987) and is involved in the central control of gastrointestinal and cardiovascular systems. In the rat, i.v. injections of 5-$HT_{1A,2 \text{ and } 3}$ receptor antagonists blocked nucleus raphe obscurus-evoked vagally-mediated increases in intragastric pressure (Krowicki & Hornby, 1993). Pancreatic DVMs have also been shown to be innervated by immunocytochemically labelled 5-HT neurones in the gigantocellular reticular nucleus and caudal raphe nuclei (Loewy *et al.*, 1994). In addition, microinjections of 5-HT into the DMN produced a decrease in heart rate (Sporton *et al.*, 1991) and augmented thyrotropin-releasing hormone-induced gastric acid secretion through a 5-$HT_{2/1C}$ receptor (McTigue *et al.*, 1992; Yoneda & Tache, 1992).

At the cellular level, application of 5-HT elicits an increase in ongoing discharge and current-pulse evoked excitability suggesting that 5-HT has an excitatory effect on DVMs (Brooks, 1991). Increased spontaneous activity is also elicited by the selective 5-HT_3 receptor agonists, 2-methyl-5-HT and 1-phenyl-biguanide and the putative 5-HT_4 receptor agonist, 5-methoxytryptamine (5-MEOT; Travagli & Gillis, 1992). In addition, the increase in spontaneous activity was partially antagonized by the selective $5HT_3$ receptor antagonist, MDL 72222 (Travagli, 1992). Therefore, it has recently been proposed that the excitatory effect is due to 5-HT acting on 5-HT_3 and 5-HT_4 receptors and that the excitatory action may involve the reduction in the slow Ca^{2+}-activated potassium current and/or a standing outward current (Travagli & Gillis, 1995). However, there are inconsistencies with these proposals. Firstly, 5-MEOT is a potent but non-selective agonist at 5-HT_4 receptors (Hoyer *et al.*, 1994; Eglen *et al.*, 1995) as it has been shown to act at other 5-HT receptor subtypes, particularly 5-$HT_{2A/2C}$ receptors (Watts & Cohen, 1992; Osborne *et al.*, 1993). Furthermore, 5-HT_4 receptor antagonists were not used to characterize the 5-HT receptor mediating the increase in excitability. Secondly, MDL 72222 only partially blocked the effect of 5-HT on DVMs suggesting that other receptor subtypes are involved. Finally, all these studies were carried out on spontaneously firing neurones; therefore little is known about the subthreshold effects of 5-HT on DVMs. This is essential if the role of 5-HT in modulating DVMs is to be defined, as *in vivo* studies have shown that the majority of DVMs do not spontaneously fire action potentials (Wang *et al.*, 1995b) whereas an *in vitro* study stated that most DVMs were spontaneously active (Travagli *et al.*, 1991).

The aim of the present study is to examine in greater detail the direct, postsynaptic effects of 5-HT and selective 5-HT receptor agonists and antagonists on visually identified DVMs in the rat. In order to achieve this we have used a thin brainstem slice preparation in combination with the whole-cell recording technique in current-clamp mode. A preliminary report has been published previously (Albert & Brooks, 1994).

[1] Author for correspondence.

Methods

Slice preparation

Sprague-Dawley rats (17–21 days) of either sex were killed by isofluorane vapour and pentobarbitone sodium (Sagatal; 600 mg kg^{-1}, i.p.). The brain and upper spinal cord were removed quickly and placed into ice-cold gassed artificial cerebrospinal fluid (aCSF) of the following composition (mM): NaCl 115, $NaHCO_3$ 25, KH_2PO_4 2.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KCl 2 and glucose 10, pH 7.4, 325 mOsm, equilibrated in 95% O_2:5% CO_2. The brainstem was removed at the rostral end of the cerebellum and glued to the cutting stage of a vibroslice 752/M (Campden Instruments Ltd). Transverse slices (175–200 μm), containing the dorsal vagal complex, were cut around the level of obex. The slices were stored in gassed aCSF at room temperature (24–25°C). A single slice was placed in a recording chamber (volume 0.5 ml), submerged under a net and continuously superfused with gassed aCSF (3–6 ml min^{-1}) at room temperature.

The time delay for drug application to the bath due to perfusion dead space was investigated using aCSF containing 30 mM KCl. KCl evoked a fast depolarization after 1 min, which reached a steady-state value within approximately 20 s. All figures showing drug applications are corrected for the 1 min delay.

Recording techniques

Patch electrodes (2–5 MΩ) were pulled from thin wall filament glass on a Brown-Flaming puller (model P-87). They were filled with an intracellular solution of the following composition (mM): K gluconate 145, $MgCl_2$ 2, $CaCl_2$ 0.1, HEPES 5, EGTA 1.1 and K_2ATP 5 (pH 7.2), 310 mOsm.

Vagal neurones were identified as clusters of fusiform shaped cells between 20–30 μm in diameter lying ventral to the nucleus tractus solitarius, using a 40× water immersion lens (Optiphot-2, Nikon).

Seals >1 GΩ were obtained before whole-cell recording was attempted in current clamp mode. Recordings were made with an Axoclamp 2A amplifier (Axon Instruments) in bridge mode to compensate for series resistance which was stable during all experiments.

To prevent ongoing action potentials, cells were held at a membrane potential (Vm) of −60 mV by injecting d.c. current through the recording electrode. R_m was monitored by voltage deflections in response to hyperpolarizing current pulses (−20 pA) of sufficient duration to reach steady-state (0.5–1 s). A series of depolarizing and hyperpolarizing current pulses (+60 to −120 pA; 0.5–1 s) was applied to measure R_m before and during responses after the membrane potential had been returned to the control level with d.c. current. At least two voltage deflections were averaged for each point on the current-voltage relationship.

Data analysis

Data were displayed on a chart recorder (PAR 1000, TDM) and stored on a FM tape-recorder (Racal). Data were also digitized using an analog-digital converter (CED 1401) for on and off-line analysis (Dr J. Dempster analysis package, Strathclyde). Statistical differences were calculated using Student's t test. The significance level was set at $P<0.05$ unless otherwise stated. All mean values are expressed as ±s.e.mean.

Drugs

All drugs were applied in the perfusate at known concentrations from stock solutions stored at −20°C. Antagonists were applied for incubation periods of 2–60 min before applying the agonist and antagonist together. 5-Hydroxytryptamine hydrochloride (5-HT), α-methyl-5-HT, ketanserin tartrate, LY 53,857 maleate (4-isopropyl-7 methyl-9-(2-hydroxy-1-methyl-propoxy carbonyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-fg] quinoline maleate), spiperone hydrochloride, 5-carboxamidotryptamine (5-CT), 5-methyoxytryptamine (5-MEOT), pindobind.5-HT_{1A}, MDL 72222 (3-tropanyl-3,5-dichlorobenzoate), bicuculline methochloride (Bic, CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) and AP-5 ((±)-2-amino-5-phosphonovaleric acid) were obtained from Research Biochemicals Inc. ICS-205-930 (1αH,3α,5αH-tropan-3-yl 1H-indole-3-carboxylate) was a gift from Dr. R. Hof, Sandoz, Switzerland. GR113808 was a gift from Glaxo, UK. All other compounds were from Sigma.

Results

Whole-cell recordings were made from 142 visually identified DVMs. Electrophysiologically, the DVMs were found to have similar properties to those described previously (Sah & McLachlan, 1992; Travagli & Gillis, 1994) with ongoing action potentials, spontaneous postsynaptic potentials (spont. p.s.ps) mainly depolarizing in nature, a prolonged after-hyperpolarization following a single action potential often lasting over 500 ms, and a mean R_m of 648±19 MΩ ($n=122$). Neurones were only analysed if they had the above characteristics, together with overshooting action potentials.

Effects of 5-HT on dorsal vagal neurones

Applications of 5-HT (1–50 μM) for 2 min elicited dose-dependent depolarizations (Figure 1a) in 59 out of 62 cells tested, with a mean amplitude of 8.2±0.5 mV ($n=59$, 20 μM). In one of the remaining cells, 5-HT (20 μM) elicited a biphasic response composed of an initial hyperpolarization followed by a depolarization (Figure 1b) while the other 2 did not respond to 5-HT. Two minute applications of 20 μM 5-HT were used routinely throughout this study since this concentration produced depolarizations large enough to enable accurate pharmacological manipulations to be carried out. The depolarizations were slow and prolonged, taking nearly 1 min from onset to reach peak amplitude (46±2 s, $n=59$) and lasting on average for over 4 min (274±8 s, $n=59$). They were reproducible and did not exhibit noticeable desensitization or run-down during recordings of up to 2 h (Figure 1a). Often the depolarization was large enough to reach threshold for action potential generation (Figure 1a). In addition, applications of 5-HT increased the frequency of postsynaptic potentials (p.s.ps) with most being depolarizing in nature (Figure 1c). This was observed as an increase in baseline noise. In the majority of DVMs (68%) the depolarizing effect of 5-HT was accompanied by an increase in apparent R_m (22.7±2.2%). The remaining DVMs either responded to 5-HT with no change (18%) or a decrease (14%) in apparent R_m (10.6±1.2%).

The slow depolarizations were maintained in media which blocked synaptic transmission (Figure 2). When applied alone, TTX (1 μM) reduced the level of synaptic activity (Figure 2b) but spont. p.s.ps were abolished only when CNQX (10 μM), AP-5 (50 μM) and bicuculline (10 μM) were also applied in the perfusate (Figure 2c). In this synaptic block medium, the R_m of the neurones (667±33 MΩ, $n=34$), amplitude of the 5-HT-evoked depolarization (10.9±1.5 mV, $n=11$), onset to peak amplitude (49±4 s, $n=11$) or duration time of response (303±26 s, $n=11$) were not altered significantly compared to the values obtained in control aCSF (Figure 3). Current-voltage relationships before and after the application of 20 μM 5-HT (Figure 4) in synaptic block medium were plotted and, from the slope of the graph calculated using linear regression 5-HT increased R_m by 14.3±2.6% ($n=8$). The reversal potential (E_{rev}) could be ascertained from the crossover points of a number of the current-voltage relationships and was calculated to be −91±1 mV ($n=4$). This is close to the E_K calculated from the Nernst equation (−89 mV, 4.2 mM $[K^+]_o$). Increasing the $[K^+]_o$ to 8.2 mM shifted the E_{rev} to −71±2 mV ($n=4$), reflecting the change in the E_K value calculated from the Nernst equation (−73 mV).

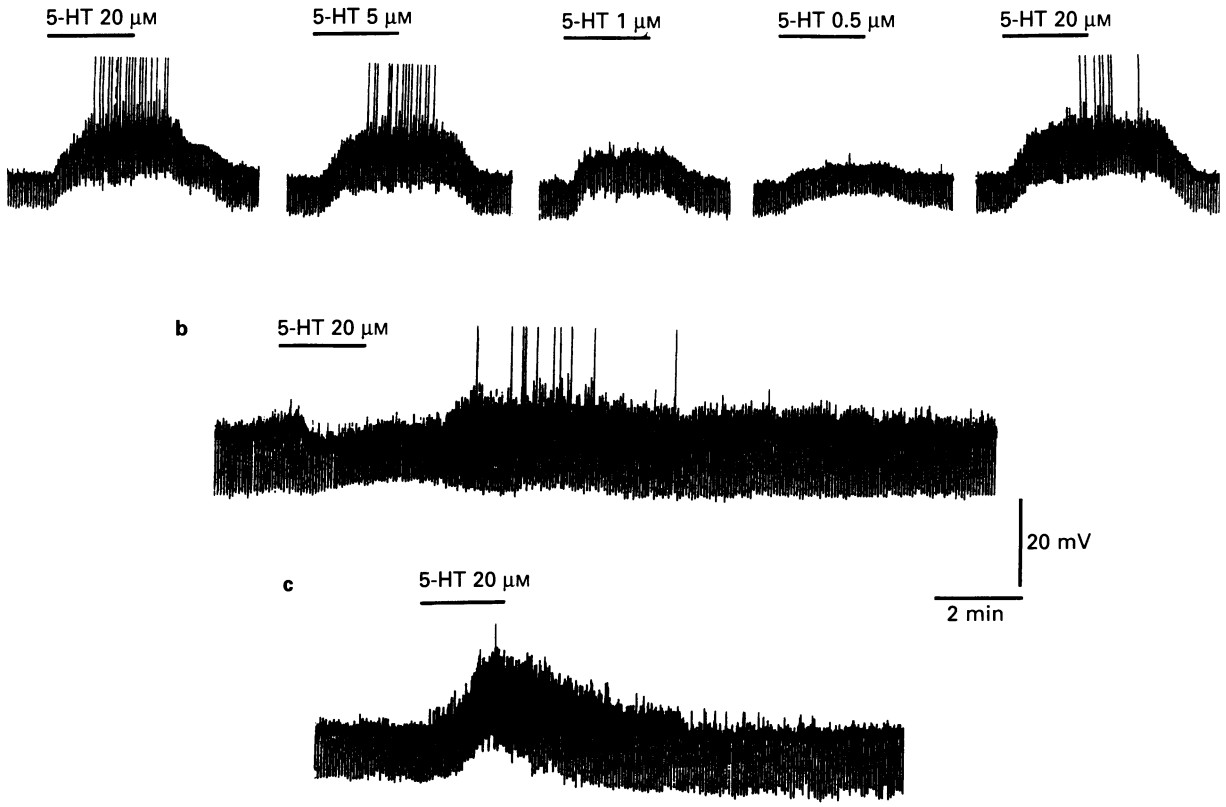

Figure 1 Effect of 5-HT on three dorsal vagal preganglionic neurones (a) 5-HT elicited a dose-dependent slow depolarization which did not exhibit any desensitization or run down. The initial application of 20 μM 5-HT was carried out less than 1 min after whole-cell configuration was obtained. The final dose of 20 μM 5-HT was applied after over 1 h of recording. Action potentials were truncated by the chart recorder. In this and other figures the downward deflections represent voltage responses to 1 s duration current pulses (−20 pA) applied every 4 s. (b) In one cell 20 μM 5-HT elicited a biphasic response, evoking an initial hyperpolarization followed by a depolarization. (c) Application of 20 μM 5-HT evoked a depolarization accompanied by a large increase in p.s.ps, the majority being depolarizing in nature.

Effects of selective 5-HT receptor antagonists

Selective 5-HT antagonists were applied to identify the receptor subtype(s) involved in the excitatory response. These were superfused at comparable concentrations and incubation times to those previously used to discriminate between 5-HT subtypes (Wang & Dun, 1990; Araneda & Andrade, 1991; Larkman & Kelly, 1992; Elliot & Wallis, 1992; Johnston *et al.*, 1993; Bobker, 1994; Torres *et al.*, 1994). Ketanserin (1 μM), a selective 5-$HT_{2A/2C}$ receptor antagonist, inhibited the depolarizing effect of 5-HT in all neurones tested to 7.1±4.5% of control responses (i.e. 7.6±1.5 mV to 0.5±0.3 mV, $n=5$, Figures 5a, 8). The structurally unrelated 5-$HT_{2A/2C}$ antagonist, LY 53,857 (1 μM), also attenuated the effect of 5-HT in all neurones tested to 19.1±7.5% of control responses (5.1±1.1 mV to 0.8±0.3 mV, $n=4$, Figures 5b, 8). In addition, the 5-$HT_{1A/2A}$ antagonist spiperone (1 μM) inhibited the effect of 5-HT to 7.5±7.4% of control values (4.2±0.7 mV to 0.3±0.3 mV, $n=5$, Figures 5c, 8) whereas the selective 5-HT_A antagonist, pindobind.5-HT_{1A} (5 μM), had no effect (not shown, $n=3$). Recovery from the application of ketanserin, LY 53,857 and spiperone even after 1 h wash-out was never complete and often, due to the constraints of the experiment, the effects were irreversible.

Selective 5-HT_3 receptor antagonists partially reduced the depolarization evoked by 20 μM 5-HT, MDL 72222 (10 μM, 85.1±3.8% of control response, 4.7±1.7 mV to 4±1.5 mV, $n=9$, Figures 6a, 8) and ICS-205-930 (1 μM, 86.3±3.1% of control response, 6.4±1.9 mV to 5.2±1.3 mV, $n=3$, Figures 6b, 8). Higher concentrations of ICS-205-930 increased this reduction although, the attenuation was not statistically significant (10 μM, 70.8±5.8% of control response, 5.6±1.7 mV to 4±0.4 mV, $n=4$, Figures 6c, 8). The selective 5-HT_4 antagonist, GR113808 (1 μM), had no effect on the depolarizing effect of 5-HT ($n=3$, Figure 7a). There was no noticeable change in Vm, R_m or level of spont. p.s.ps activity with all the antagonists tested.

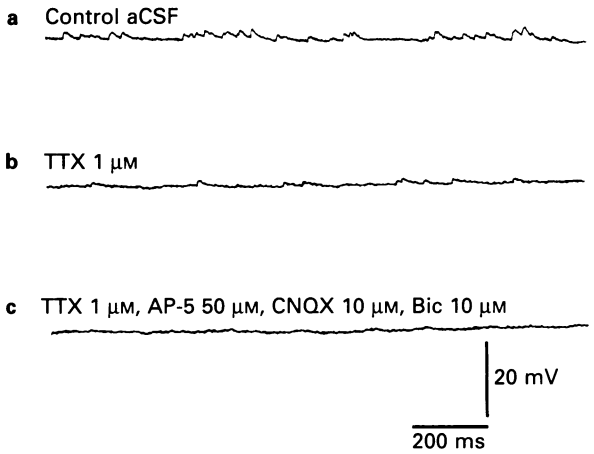

Figure 2 TTX-resistant spontaneous p.s.ps are blocked by excitatory and inhibitory amino acid antagonists: (a) Spontaneous p.s.p. events mainly depolarizing in nature are present in control aCSF; (b) the frequency of the spontaneous p.s.ps in the same motoneurone is reduced but not completely blocked in aCSF containing 1 μM TTX; (c) the spontaneous p.s.ps are attenuated in a medium containing 1 μM TTX, 10 μM CNQX, 50 μM AP-5, 10 μM Bic.

Effects of selective 5-HT receptor agonists

To elucidate further the receptor subtype mediating the slow depolarization, selective 5-HT receptor agonists were applied in the perfusate. The selective 5-$HT_{2A/2C}$ agonist, α-methyl-5-HT, mimicked the effect of 5-HT (Figure 9a), causing a depolarization of mean amplitude 8.9±0.6 mV (n=12, 50 μM, 2 min) with an onset to peak amplitude of 60±4 s (n=12, 50 μM) and response duration of over 5 min (mean=328±28 s, n=12, 50 μM). In addition, α-methyl-5-HT increased both R_m and the occurrence of p.s.ps. The depolarization and increase in R_m were maintained in synaptic block medium and were blocked by ketanserin (n=2, 100% reduction of control response). In synaptic block medium, current-voltage plots showed a α-methyl-5-HT increased R_m by 13.2±2% (n=5, 50 μM) with a E_{rev} of −90±2 mV (n=4). Applications of the 5-HT_1 receptor agonist, 5-CT and the putative 5-HT_4 receptor agonist, 5-MEOT for 2 min also mimicked the effect of 5-HT, evoking slow depolarizations (5-CT: 7.3±1.1 mV, n=12, 20 μM, : 5-MEOT; 6.6±0.8 mV, n=13, 50 μM, Figures 9b, c) which were maintained in synaptic block medium and inhibited by ketanserin (5-CT; 2.8±2.7% of control response, 8±1.5 mV to 0.3±0.2 mV, n=4: 5-MEOT; 10.1±1.2% of control response, 6.3±0.9 mV to 0.7±0.2 mV, n=3). In one cell tested, prolonged application of the selective 5-HT_4 antagonist, GR113808 did not block 5-MEOT mimicking the response to 5-HT (Figure 7b). The selective 5-HT_3 agonist, 2-methyl-5-HT (100–300 μM), excited 5 out of 9 cells tested. This response consisted of a depolarization (3.8±0.6 mV, n=3, 100 μM) associated with a decrease in apparent R_m (17.4±2.2%) and/or an increase in p.s.ps (Figure 9a).

Discussion

The effect of 5-HT on DVMs was studied using a wide range of selective 5-HT agonists and antagonists. The results provide for the first time, evidence that the excitatory action of 5-HT on DVMs is predominantly mediated by 5-HT_{2A} receptors and not by 5-HT_4 receptors as recently proposed (Travagli *et al.*, 1992).

Of the cells tested, 95% produced a comparable slow and prolonged dose-dependent depolarization in response to 5-HT which was capable of reaching threshold for initiating action potentials. The excitatory response of DVMs to 5-HT did not exhibit any desensitization or run-down when repeated applications of 5-HT were made. This suggests that either intracellular component wash-out did not occur or that intracellular component wash-out did not affect the regulation of the 5-HT receptors involved in the response. Therefore, it is appropriate to use the whole-cell recording technique to study the subthreshold effects of 5-HT in this preparation. Although 5-HT (1–10 μM) elicited depolarizations, these were not of sufficient amplitude to allow accurate pharmacological manipulations to be carried out. Therefore, in order to examine the effects of antagonists, 5-HT was used at 20 μM since at this dose it elicited a depolarization of satisfactory amplitude. The 20 μM concentration of 5-HT used in the present study may explain the higher proportion of DVMs excited (95%) compared to a previous one (65%) which used 0.3–1 μM 5-HT (Travagli & Gillis, 1995).

5-HT evoked an increase in p.s.ps which were mainly depolarizing in nature, although this may be due to DVMs being held at −60 mV, which was close to E_{Cl}, and reduced the amplitude of hyperpolarizing events. The increase in 5-HT-evoked p.s.ps probably represents indirect effects on interneurones within the DMN and neurones of the nucleus tractus solitarius (NTS) where 5-HT-containing nerve terminals are also present (Steinbusch, 1981). The slow depolarization was mediated by a direct, postsynaptic effect of 5-HT on DVMs. This is proven by the maintenance of the depolarization during the perfusion with a synaptic block medium which, not only prevents action potentials but, inhibits TTX-resistant excitatory and inhibitory spont. p.s.ps (Travagli, 1991).

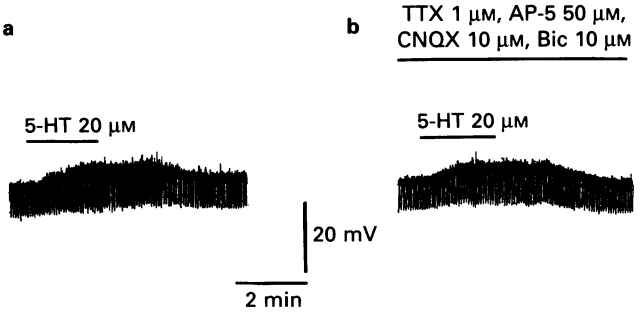

Figure 3 The depolarizing effect of 5-HT is maintained in synaptic block medium: (a) 5-HT applied in control aCSF elicited a slow depolarization and an increase in p.s.ps; (b) the response to 5-HT was maintained in the same cell during superfusion of a synaptic block medium containing TTX 1 μM, CNQX 10 μM, AP-5 50 μM, Bic 10 μM.

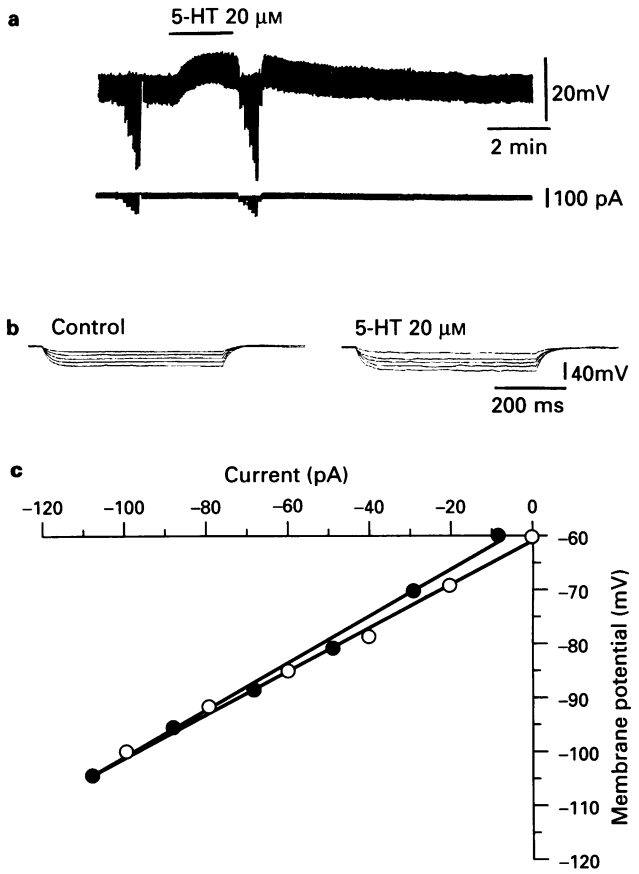

Figure 4 The excitatory effect of 5-HT is associated with an increase in R_m. (a) Hyperpolarizing current pulses of increasing amplitude were applied to a dorsal vagal preganglionic neurone before and during an application of 20 μM 5-HT in synaptic block medium. A holding current of −8.79 pA was applied during the response to return the membrane potential to its control level of −60 mV. (b) Using a faster time course, voltage deflections from the above data are shown to increase in the presence of 5-HT demonstrating that the response is associated with an increase in R_m. (c) From the data provided by (a), current-voltage relationships were plotted for before (○) and during (●) the 5-HT response. Using linear regression analysis 5-HT was calculated to increase the gradient from 393 MΩ to 438 MΩ. This corresponds to an increase in R_m of 11.5%. The crossover of the control and 5-HT lines indicates that the E_{rev} is −92 mV.

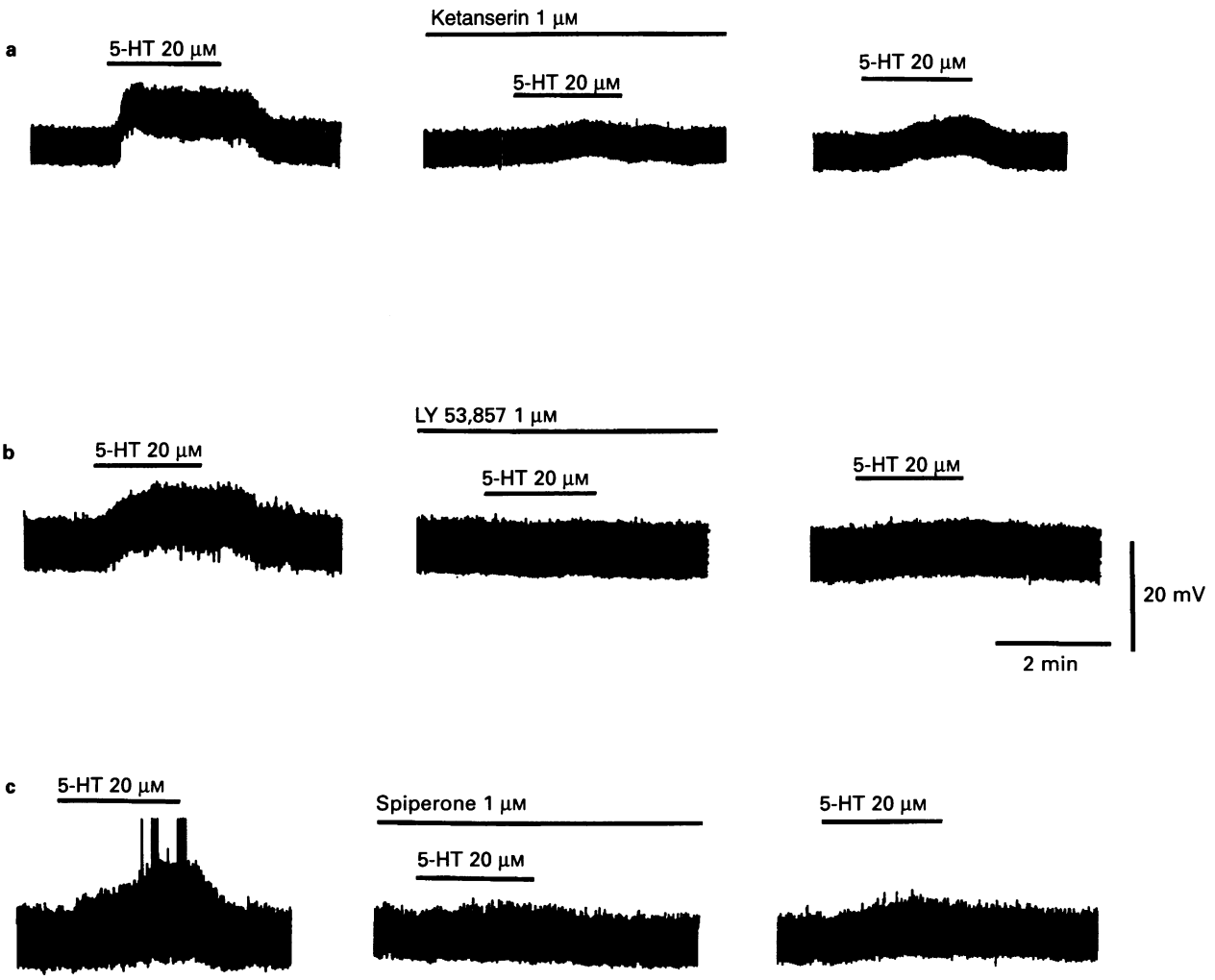

Figure 5 The effect of selective 5-$HT_{2A/2C}$ receptor antagonists on the excitatory response to 5-HT on three different dorsal vagal preganglionic neurones (DVMs). (a) Ketanserin (1 μM) attenuates the effect of 5-HT after a preincubation period of 50 min. Partial recovery was observed in this cell after over 30 min wash-out. (b) LY 53,857 (1 μM) blocked the response to 5-HT after a 30 min preincubation period. A small recovery response was observed after a prolonged wash-out period of over 1 h. (c) Spiperone (1 μM) also attenuated the effect of 5-HT on a DVM after a 15 min preincubation period. Again, partial recovery was obtained after 30 min wash-out.

The application of a selective 5-HT antagonist did not noticeably alter Vm, R_m or spont.p.s.ps. activity suggesting there is no tonic release of 5-HT occurring in the brainstem slice preparation, although in this study quantitative analysis was not performed to provide a definitive answer.

The mechanism of 5-HT action was investigated by measuring its effect of R_m. In the majority of DVMs (68%), 5-HT evoked an increase in apparent R_m. However, in a proportion of DVMs 5-HT either elicited a decrease in apparent R_m (14%) or no change (18%). In synaptic block medium the effect of 5-HT on R_m was measured by returning the depolarized Vm to -60 mV with negative d.c. holding current. Under these conditions, 5-HT increased R_m with a E_{rev} correlated to the $[K^+]_o$, providing evidence that the mechanism of the slow depolarization is via the closure of K^+ channels. However, not all DVMs exhibited a E_{rev}. Combining this evidence with the observations that in a number of DVMs 5-HT evoked either a decrease or no change in R_m it is possible that the closure of K^+ channels is not the only mechanism underlying the slow depolarization. For instance, 5-HT has been shown to augment the non-selective cation current (I_h; Bobker & Williams, 1991; Larkman & Kelly, 1992) which is present in DVMs (Travagli and Gillis, 1994). This could account for the small changes in conductance and, in neurones where the closure of K^+ channels is not predominant, the lack of a E_{rev}. To elucidate the nature of the ionic currents involved, greater detailed examination needs to be carried out, although from previous studies (Rasmussen & Aghajanian, 1990; Elliot & Wallis, 1992) it could be hypothesized that 5-HT, in part, closes K^+ leak channels which are open at the control Vm of -60 mV.

Many studies have shown previously that an array of different neuronal types respond to 5-HT with a slow depolarization via a 5-HT_2 receptor-mediated pathway linked to the closure of a K^+ channel (North & Uchimura, 1989; Wang & Dun, 1990; Araneda & Andrade, 1991; Larkman & Kelly, 1991; Elliot & Wallis, 1992; Johnston *et al.*, 1993; Bobker, 1994; Pickering *et al.*, 1994). The evidence for 5-HT_2 receptor-mediation in the present study is (i) the response to 5-HT was slow and prolonged suggesting the involvement of a second messenger system; (ii) ketanserin and LY 53,857 both consistently blocked the response to 5-HT whereas MDL 72222, ICS-205-930, pindobind.5-HT_{1A} and GR113808 did not and, (iii) α-methyl-5-HT mimicked the direct, postsynaptic effects of 5-HT by eliciting a slow depolarization accompanied by an increase in R_m which were both maintained in synaptic block medium and blocked by ketanserin. In addition, the 5-HT_1 receptor agonist, 5-CT and the putative 5-HT_4 receptor agonist, 5-MEOT, which both have been shown to act with low potency at 5-HT_2 receptors (Watts & Cohen, 1992; Osborne *et al.*, 1993; Pickering *et al.*, 1994), mimicked the effect of 5-HT.

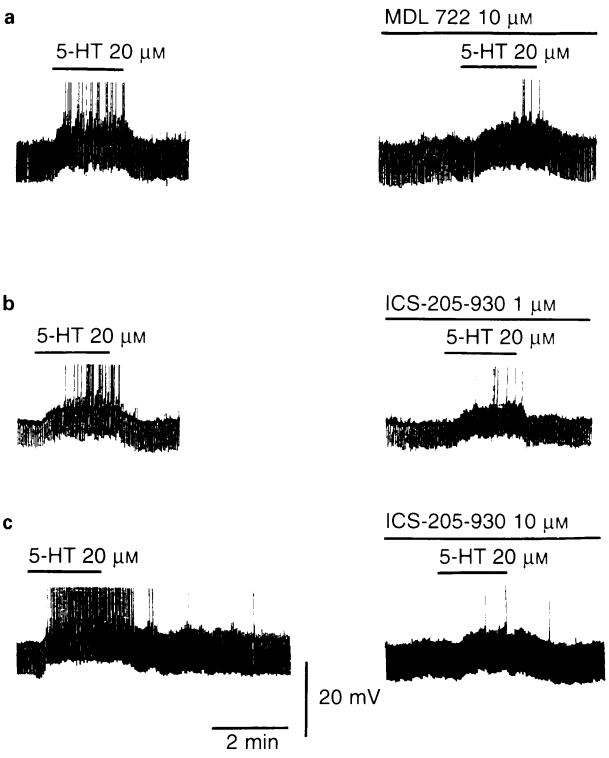

Figure 6 The effect of selective 5-HT_3 and 5-HT_4 receptor antagonists on three different dorsal vagal preganglionic neurones. (a) Superfusion of 10 μM MDL 72222 produced a small reduction on the response to 20 μM 5-HT after a 15 min preincubation period. (b) Superfusion of 1 μM ICS-205-930 produced minor inhibition on the effect to 5-HT after 20 min preincubation. (c) At a higher concentration of ICS-205-930 (10 μM) the response to 5-HT was attenuated after 25 min preincubation although the % reduction was insignificant ($P > 0.05$).

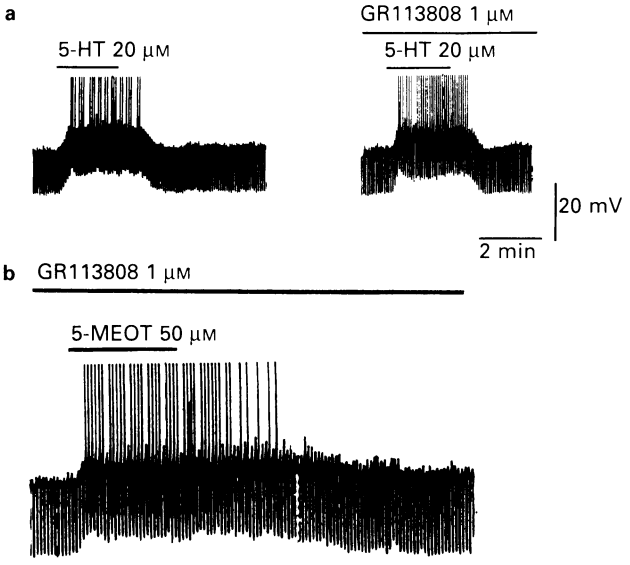

Figure 7 The effect of a selective 5-HT_4 receptor antagonist on the depolarizing response of 5-HT and 5-MEOT on a dorsal vagal preganglionic neurone (DVM). (a) The effect of 5-HT on a DVM was not attenuated by the selective 5-HT_4 receptor antagonist, GR113808 after a 40 min preincubation period. (b) In the same DVM as (a), the putative 5-HT_4 receptor agonist, 5-MEOT, still mimicked the effect of 5-HT after GR113808 (1 μM) had been continually superfused for over 1 h.

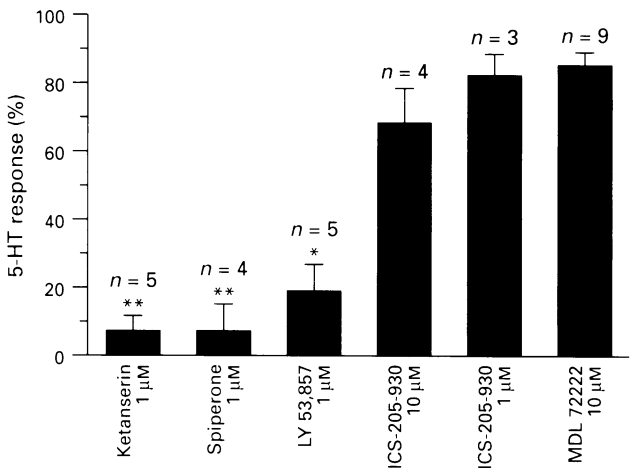

Figure 8 A chart showing the % reduction of control depolarizing responses to 5-HT by various selective 5-HT receptor subtype antagonists. Ketanserin, spiperone and LY 53,857 all significantly inhibited the control responses. $*P < 0.05$ and $**P < 0.01$.

Two closely related subtypes, both in sequence homology and pharmacolgical profile, of the 5-HT_2 receptor family are found in the mammalian central nervous system; 5-HT_{2A} and 5-HT_{2C} (Hoyer *et al.*, 1994). Our results provide powerful evidence for a postsynaptic action of 5-HT acting at the 5-HT_{2A} subtype due to the attenuating action of spiperone, which is 1000 times more selective for 5-HT_{2A} than 5-HT_{2C} receptors (Peroutka, 1994). This corresponds to recent evidence suggesting a high level of 5-HT_{2A} receptor mRNA present in the dorsal motor nucleus of the vagus of the rat (Pompeiano *et al.*, 1994; Wright *et al.*, 1995) and also, to *in vivo* work where 5-HT increases spontaneous activity of antidromically identified DVMs via a 5-$HT_{2A\,2C}$ receptor (Wang *et al.*, 1995a).

The selective 5-HT_3 receptor antagonists MDL 72222 and ICS-205-930 partially reduced the response to 5-HT. Although the effect of 5-HT_3 receptor antagonists was not statistically significant a similar reduction of comparable amplitude was observed in all neurones tested. This reduction combined with the increase in excitability evoked by 2-methyl-5-HT does suggest that 5-HT_3 receptors contribute to the effect of 5-HT on DVMs. This is in agreement with *in vitro* and *in vivo* studies (Travagli *et al.*, 1992; Wang *et al.*, 1995b). However, it has not been determined whether this effect is a direct, postsynaptic one, a presynaptic action or a combination of both effects as observed within the NTS (Glaum *et al.*, 1992). In the rat, autoradiographic studies have shown specific binding of 5-HT_3 receptor ligands in the DMN (Steward *et al.*, 1993) although recent data in mice has revealed a very low level of 5-HT_3 receptor mRNA in this area (Tecott *et al.*, 1993). This suggests that the majority of 5-HT_3 receptors are located presynaptically.

There are several reasons why it is unlikely that 5-HT_4 receptors are involved in the effect of 5-HT on DVMs as previously suggested (Travagli & Gillis, 1992). (i) The selective 5-HT_4 antagonist GR113808, which has been shown to block potently the well-characterized 5-HT_4 receptor effect in the hippocampus (Torres *et al.*, 1994), had no effect on the depolarizing effect of 5-HT on DVMs. (ii) The direct, postsynaptic depolarizing effect of 5-MEOT, a potent but nonselective 5-HT_4 receptor agonist (Eglen *et al.*, 1995), on DVMs was attenuated by the selective 5-$HT_{2A\,2C}$ receptor antagonist, ketanserin and not by GR113808 and, (iii) recent evidence from *in situ* hybridization experiments suggest that there is a very low level of 5-HT_4 receptor mRNA within the rat brainstem (Eglen *et al.*, 1995; Vilaro *et al.*, 1995). Furthermore, ICS-205-930 at a concentration (10 μM) previously shown to an-

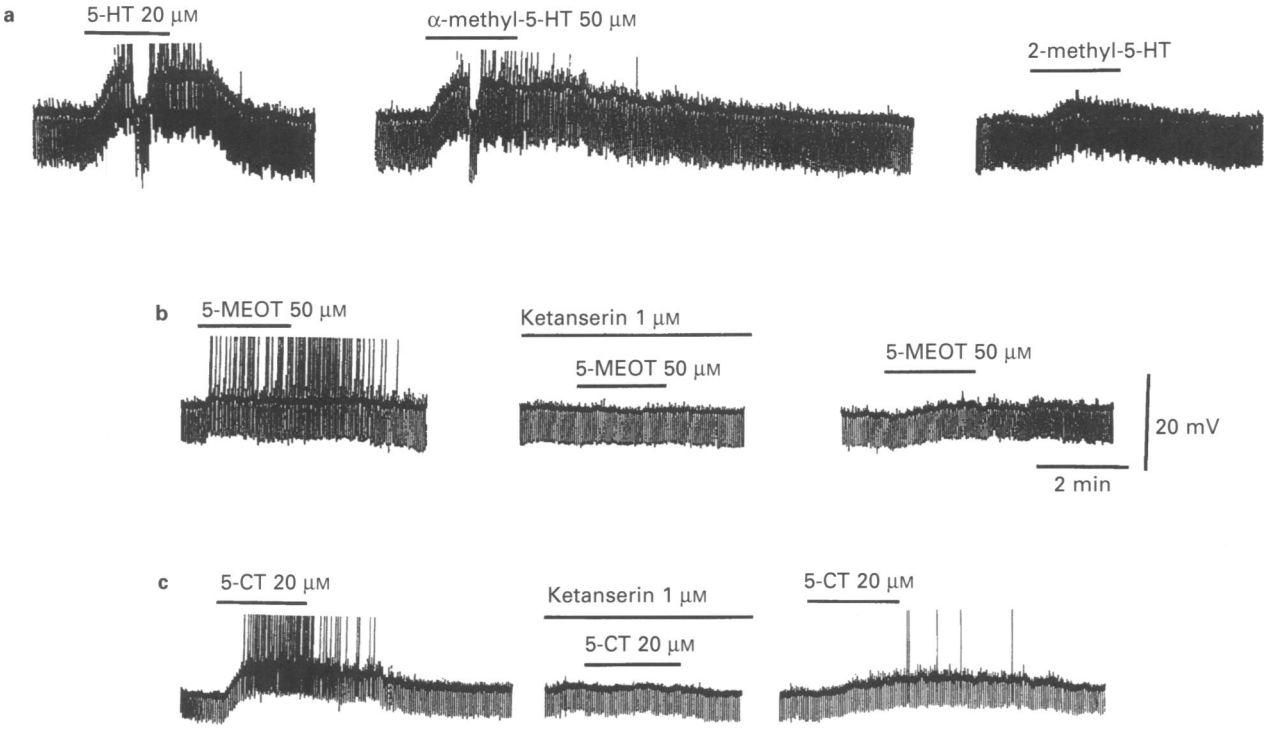

Figure 9 The effect of selective 5-HT receptor agonists on dorsal vagal preganglionic neurones (DVMs). (a) Application of 50 μM α-methyl-5-HT mimicked the effect of 5-HT but the overall response was prolonged. In the same cell, 100 μM 2-methyl-5-HT evoked a small depolarization with an increase in p.s.ps. (b) Application of 50 μM 5-MEOT evoked an excitatory effect on a DVM which was blocked by ketanserin after a preincubation period of 15 min. Partial recovery was observed after 40 min wash-out. (c) Application of 20 μM 5-CT also elicited an excitatory effect on another DVM which was attenuated by ketanserin after a 15 min preincubation period. Partial recovery was observed after over 1 h wash-out.

tagonize 5-HT_3 and 5-HT_4 receptors (Bockaert *et al.*, 1990), only partially antagonized the response to 5-HT. This insignificant attenuation may therefore reflect only the 5-HT_3 component of the response or the pharmacological profile of the 5-HT_{2A} subtype found on DVMs. This is comparable to the 5-HT receptor characterized on a guinea-pig trachea preparation which was activated by 5-MEOT and blocked by ICS-205-930 at high concentrations (Watts & Cohen, 1992).

This study provides the first detailed pharmacological profile of the effect of 5-HT on dorsal vagal neurones, *in vitro*. In conclusion, 5-HT elicits a strong excitatory effect on 95% of DVMs. The properties of this response are consistent with 5-HT exerting a direct postsynaptic action through 5-HT_{2A} receptors due, in part, to a reduction in K^+ conductance. From the large proportion of neurones excited by 5-HT and the suggested origin from caudal raphe nuclei, it is probable that 5-HT plays an important modulatory role in regulating vagal output involved in the control of various autonomic functions during different behavioural states.

This study was supported by the Wellcome Trust. A.P.A. is a recipient of a Wellcome Prize Studentship. The comments of Dr D. Jordan, Dr M. Gilbey and Dr S. Deuchars on the manuscript are gratefully acknowledged.

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(Received June 6, 1995
Revised January 15, 1996
Accepted July 2, 1996)