Psychopharmacology (1989) 98:45–50

Psychopharmacology

Ritanserin, a 5-HT_2 receptor antagonist, activates midbrain dopamine neurons by blocking serotonergic inhibition

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Abstract. The effect of systemic administration of ritanserin (R 55667), a 5-hydroxytryptamine (5-HT_2) receptor antagonist, on midbrain dopamine (DA) neurons was studied with single cell recording techniques in the chloral hydrate anesthetized male rat. Dopamine cells of the zona compacta, substantia nigra (ZC-SN) and the ventral tegmental area (VTA) were identified by established criteria. Ritanserin (0.5–2.0 mg/kg, IV) dose-dependently increased both the burst firing and firing rate of the midbrain DA neurons. These effects were prevented by endogenous 5-HT depletion through pretreatment with the 5-HT synthesis inhibitor para-chlorophenylalanine (PCPA, 300 mg/kg, IP, ×3), which did not significantly alter the firing characteristics of the midbrain DA cells when given alone. These results suggest that 5-HT exerts an inhibitory control of midbrain DA cell activity mediated by 5-HT_2 receptors. The stimulatory effect of ritanserin on midbrain DA systems might contribute to some of its clinical effects, such as improvement of mood, drive and motivation as well as its therapeutic actions in parkinsonism and type II schizophrenia.

Key words: Dopamine – Ritanserin – Serotonin – Single cell recording – Substantia nigra – Ventral tegmental area

Ritanserin, a selective antagonist at serotonin (5-hydroxytryptamine, 5-HT) receptors of the 5-HT_2 (S_2) type with low affinity for dopamine (DA) D_2 receptors (Leysen et al. 1985), has recently been reported to exert significant therapeutic effects in several neuropsychiatric disorders. For example, a clearcut mood elevating action in dysthymic states has been observed (Reyntjens et al. 1986), including improvement of fatigue, drive and motivation. In addition, ritanserin has been found to reduce negative symptoms in chronic schizophrenia (Gelders et al. 1986; Reyntjens et al. 1986) as well as extrapyramidal side effects such as parkinsonism, associated with neuroleptic treatment (Bersani et al. 1986). Other studies claim a beneficial effect of ritanserin in Parkinson's disease (Maertens de Noordhout and Delwaide 1986; Meco et al. 1986). Thus, the mode of action of specific 5-HT_2 receptor antagonists in neuropsychiatric disorders is of considerable interest, especially since postmortem studies indicate changes in brain 5-HT_2 receptor densities in schizophrenic patients (Mita et al. 1986), patients with Alzheimer's disease (Crow et al. 1984; Reynolds et al. 1984) and suicide victims (Stanley and Mann 1983). Yet, the functional and pharmacological significance of such clinical observations remains to be clarified.

The distribution of specific 5-HT_2 binding sites appears to be similar in rat and human brain (Pazos et al. 1985, 1987). Animal experiments have indicated that central 5-HT_2 receptors are involved in the mediation of behavioural effects of 5-HT such as the 5-hydroxytryptophan-induced head twitch (see Leysen 1984). Also, these receptors have been ascribed an important rôle in major effects of hallucinogens (Glennon et al. 1983; Rasmussen and Aghajanian 1986), which are antagonized by ritanserin in rats (Colpaert et al. 1985). Finally, electrophysiological experiments have described a slow depolarization of central neurons apparently mediated by 5-HT_2 receptors (Aghajanian 1981; Davies et al. 1987).

Major clinical actions of ritanserin, such as enhanced mood and motivation as well as antiparkinsonian effects, might suggest a facilitatory effect of the drug on brain dopaminergic neurotransmission. Studies utilizing histochemistry reveals both 5-HT and tryptophan hydroxylase activity within the midbrain DA cell clusters (Fuxe 1965; Reubi and Emson 1978; Steinbusch 1981) and autoradiography shows 5-HT-containing projections from the midbrain raphe nuclei to the substantia nigra (SN; Fibiger and Miller 1977; Imai et al. 1986) and to the adjacent ventral tegmental area (VTA; Parent et al. 1981). Recently, serotonergic terminals have been shown to make direct synaptic contact with DA cells in the SN and VTA (Hervé et al. 1987; Nedergaard et al. 1988a). In addition, Dray et al. (1976) found that stimulation of neurons in the median raphe nucleus produces a marked inhibition of SN neurons, a response which is prevented by endogenous 5-HT depletion through pretreatment with para-chlorophenylalanine (PCPA; Fibiger and Miller 1977).

Consequently, the present study was undertaken to explore the effect of systemic ritanserin administration on the activity of midbrain DA neurons in the zona compacta (ZC), SN and the VTA with single cell recording techniques. Furthermore, we tested whether the stimulatory effect observed could be antagonized by 5-HT depletion by repeated pretreatment with PCPA.

Preliminary results were presented at the Society for Neuroscience Annual Meeting in New Orleans 1987 (Ugedo et al. 1987).

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Materials and methods

Animals and preparation. Male Sprague-Dawley rats weighing 200–300 g (Alab, Sollentuna, Sweden) were used. Rats were anesthetized with chloral hydrate (400 mg/kg IP), after which a tracheal cannula and a jugular vein catheter for intravenous administration of drugs and additional anesthetic were inserted before the animal was mounted in a David Kopf stereotaxic instrument. Rectal temperature was kept at 36–37° C by means of an electric heating pad. The skull was exposed and a small hole was drilled over the ZC-SN or the VTA. Coordinates, determined from the atlas of Paxinos and Watson (1986), were 3.3 mm anterior and 2.0 mm lateral to lambda for the ZC-SN, and 3.3 mm and 0.7 mm for the VTA. In some experiments, rats were pretreated with PCPA, 300 mg/kg/day IP for 3 consecutive days and tested 24 h after the last injection.

Extracellular recording procedures. Extracellular recording electrodes were pulled in a Narishige vertical puller from Omegadot glass tubing and filled with 2 M NaCl containing 2% Pontamine Sky Blue. Electrode impedance was 2–4 MΩ measured at 135 Hz. The electrode was lowered into the brain by means of a hydraulic microdrive. Typical DA cells were found 6.5–7.5 mm from skull surface in the ZC-SN, and 7.5–8.5 mm in the VTA.

Recordings were made from cells whose electrophysiological characteristics matched those previously established for midbrain dopamine cells (Guyenet and Aghajanian 1978; Wang 1981; Grace and Bunney 1983), i.e. a triphasic action potential of more than 2 ms duration with a notch in the initial ascending portion and a basal firing rate of 1–10 Hz. In addition, the recorded cells corresponded to antidromically identified DA neurons previously described by us (Grenhoff et al. 1986).

Extracellular action potentials were amplified, discriminated and monitored on an oscilloscope and an audiomonitor. Burst firing, firing rate and regularity of firing were analysed from inter-spike time interval histograms (ISH) created by an Apple II plus computer. The ISH program collects all time intervals between action potentials and displays each interval within the appropriate time bin of the abscissa of the histogram (Gerstein 1960). In the present study, the ISH program had 256 bins with bin-widths of 1–8 ms. Burst firing was measured as the percentage ratio of spikes in bursts to the total number of spikes of an ISH. Burst onset was signalled by an interspike interval less than 80 ms and burst termination by an interval greater than 160 ms, values previously shown to be optimal (Grace and Bunney 1984). As a measure of regularity of firing the variation coefficient (v-c) was employed (Werner and Mountcastle 1963). The variation coefficient is the percentage ratio of the standard deviation to the mean interval value of an ISH. Each ISH was based on 500 consecutive spikes. For analysis of drug effect, an ISH recorded before drug administration was compared to an ISH recorded from the same cell within 5 min after drug administration. Only one cell was studied in each animal.

At the end of each experiment when suitable, the cells were tested for the inhibitory effect of a low dose of apomorphine (25 μg/kg IV). Finally a 5 μA negative current was passed through the recording electrode, leaving a blue spot at the site of the tip of the electrode. The animal was perfused transcardially with 10% formalin, the brain was removed and the recording site was verified histologically. All electrode marks were found within the ZC-SN or VTA for cells included in this study.

Drugs. *p*-Chlorophenylalanine methyl ester (Sigma, St. Louis, USA) and apomorphine HCl (Apoteksbolaget, Sweden) were dissolved in 0.9% NaCl. Ritanserin (sample for clinical trials) was generously supplied by Janssen Pharmaceutica, Beerse, Belgium.

Data analysis. Statistical evaluation of firing rates and variation coefficients was made with Student's paired *t* test. Since burst firing values deviated from a normal distribution, they were analyzed with the non-parametric Wilcoxon matched-pairs signed ranks test and no SEM values were calculated for them. A two-tailed *P* value less than 0.05 was considered significant.

Results

Firing characteristics of midbrain DA neurons

Midbrain DA cells of the ZC-SN or VTA displayed firing rates within the range of 1–10 Hz and showed generally two types of firing patterns: 1) single spike firing and 2) burst firing. Bursts were characterized as a series of 2–15 spikes of diminishing amplitude, separated by short intervals and followed by a longer pause (see Methods). A cell was defined as burst firing when it fired at least two three-spike bursts out of a series of 500 spikes. Table 1 shows the baseline firing parameters of the recorded cells.

Effects of ritanserin on DA neurons

In the ZC-SN, cumulative doses of ritanserin (0.5–2.0 mg/kg IV) increased burst firing in a dose-dependent manner (Fig. 1), an example of which is given in Fig. 2. Occasionally a non-burst firing cell was brought into a burst firing mode. At 1.0–2.0 mg/kg (IV) ritanserin also increased the firing rate (Fig. 3), which is reflected in the post-drug ISH (Fig. 2) as a shift to the left (shorter inter-spike intervals). The lowest dose produced, in addition to increased burst firing, decreased regularity of the firing pattern, quantified by a

Table 1. Firing properties of midbrain DA neurons in untreated and *para*-chlorophenylalanine (PCPA)-pretreated rats

Firing characteristics	ZC-SN		VTA	
	Untreated	PCPA	Untreated	PCPA
Rate, Hz	3.55±0.47	4.07±0.46	3.74±0.52	4.30±0.42
Burst firing %	11	9	18	21
V-C%	47.7±3.4	39.3±4.1	60.3±4.3	57.3±4.0
n	17	16	15	13

The variation coefficient (V-C) is the ratio between the standard deviation and the mean interval value of an inter-spike time interval histogram, expressed as per cent. PCPA was administered in a dose of 300 mg/kg, IP daily for 3 days. The experiments were performed 24 h after the last injection. Each value is the mean ±SEM of *n* cells per group

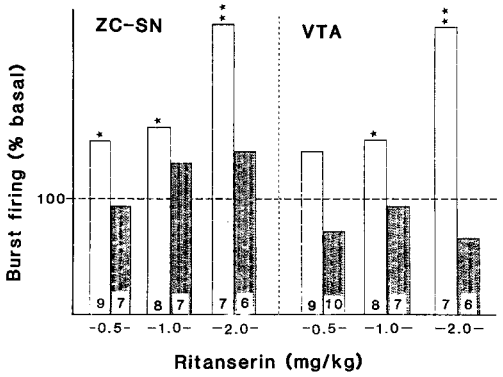

Fig. 1. Effect of cumulative doses of ritanserin (0.5–2.0 mg/kg IV) on the burst firing of ZC-SN and VTA dopamine neurons in untreated (□) and PCPA pretreated rats (300 mg/kg/day IP, 3 days) (■). Results (mean) are presented relative to control values given in Table 1. The figure on the columns indicates the number of cells. * $P<0.05$, ** $P<0.02$

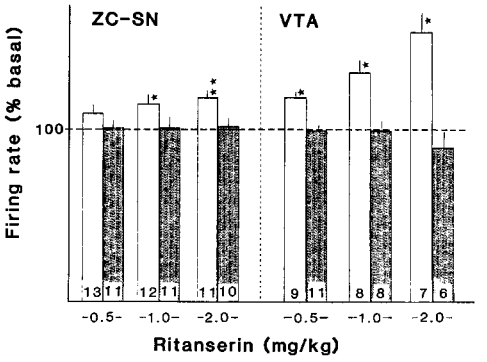

Fig. 3. Effect of cumulative doses of ritanserin (0.5–2.0 mg/kg) on the firing rate of ZC-SN and VTA dopamine neuron in untreated (□) and in PCPA pretreated rats (300 mg/kg/day IP, 3 days) (■). Results (mean ± SEM) are presented as percentage of control values given in Table 1. The figure on the column indicates the number of cells. * $P<0.05$, ** $P<0.01$

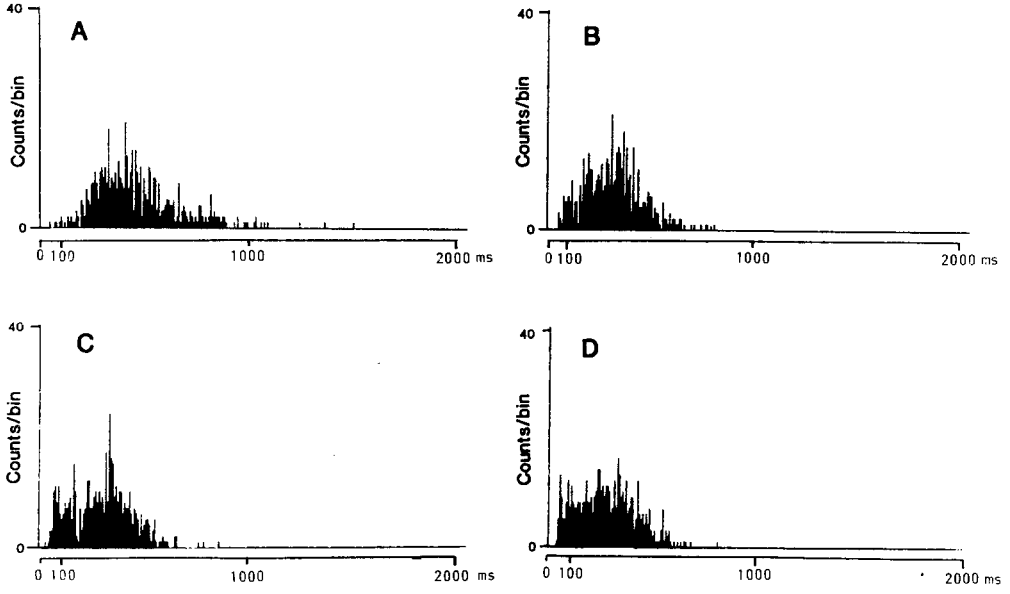

Fig. 2A–D. Inter-spike time interval histograms from a ZC-SN dopamine cell before (**A**) and after ritanserin. Ritanserin 0.5, 1.0 and 2.0 mg/kg IV (**B**, **C** and **D**) increased firing rate from 2.15 to 3.08, 3.30 and 3.48 Hz, and burst firing from 0 to 2, 8 and 13%. This is reflected in a shift to the left (shorter inter-spike intervals) of the histogram and the emergence of a bimodal interval distribution, the left peak representing the short intervals within bursts

significant increase of the variation coefficient from 48.7 ± 4.5% (mean ± SEM) to 52.8 ± 4.8% ($n=13$, $P<0.01$).

In the VTA, 1.0–2.0 mg/kg (IV) of ritanserin increased the burst firing (Fig. 1) and all doses (0.5–2.0 mg/kg IV) produced an increase in the firing rate in a dose-dependent manner (Fig. 3). No changes in regularity were observed at any dose of ritanserin in the VTA. An example of the post-drug ISH recorded from a VTA-DA cell before and after ritanserin administration is shown in Fig. 4.

In some experiments, an inhibition of cell firing was seen in the first minute after injection. This transient effect was immediately followed by the excitation described here. The excitatory action of ritanserin was never observed to abate, not even when a neuron was followed for over 4 h. This long lasting effect of ritanserin on DA neurons may reflect the slow dissociation of ritanserin from 5-HT_2 receptors observed in binding experiments (Leysen et al. 1985).

After ritanserin administration an IV injection of a low dose of apomorphine (25 μg/kg) produced a 30–100% inhibition of all cells tested ($n=12$).

Effect of ritanserin on DA cells after 5-HT depletion

In order to determine the role of endogenous 5-HT in the excitatory effect of ritanserin on DA cells, rats were pretreated with the 5-HT synthesis inhibitor PCPA, which causes a virtually complete depletion of 5-HT in brain tissue (Koe and Weissman 1966), in a dose of 300 mg/kg/day IP for 3 days. Under these conditions the baseline firing characteristics of midbrain DA cells were not significantly different from those obtained in untreated animals (Table 1). However, this pretreatment totally blocked the excitatory effect of ritanserin (0.5–2.0 mg/kg IV) on ZC-SN and VTA DA neurons (Fig. 3). At the highest dose (2.0 mg/kg IV), a decrease in regularity of the firing pattern, i.e., an increase

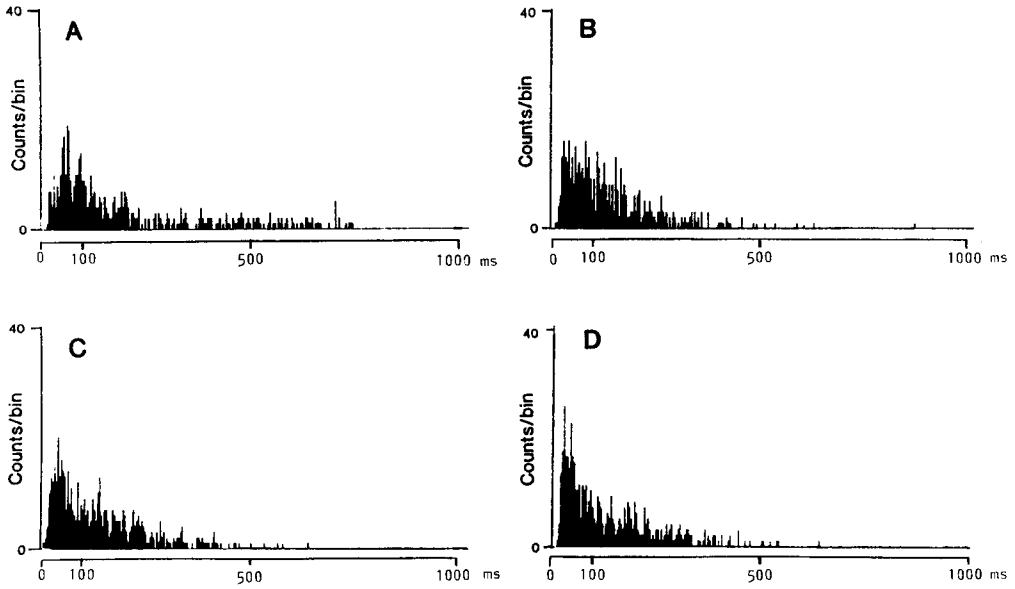

Fig. 4A–D. Inter-spike time interval histograms from a VTA dopamine cell before (**A**) and after ritanserin. Ritanserin 0.5, 1.0 and 2.0 mg/kg IV (**B**, **C** and **D**) increased the firing rate from 4.90 to 7.03, 7.04 and 7.18 Hz and the percentage of burst firing from 59 to 69, 72 and 74%. This is reflected in the shift to the left (shorter inter-spike intervals) of the histogram

in the variation coefficient from 35.9±4.3% (mean ±SEM) to 39.2±4.7%, was seen in the ZC-SN ($n=10$, $P<0.05$). Thus, a higher dose of ritanserin was necessary to elicit decreased regularity of ZC-SN cell firing after PCPA pretreatment.

The typical inhibitory effect of a low dose of apomorphine (25 µg/kg IV) on the midbrain DA neurons was always observed after ritanserin administration in the PCPA-pretreated rats ($n=10$).

Discussion

The major finding of the present investigation is the significant and long lasting, dose-dependent ritanserin-induced activation of midbrain DA neurons, both in the ZC-SN, the origin of the nigrostriatal DA system, and in the VTA, which is the source for the so-called mesolimbic DA system (see Björklund and Lindvall 1984). The increased activity of the DA cells involved both single spike firing and burst firing without a simultaneous change in regularity of discharge. Although conventional neuroleptics increase DA cell firing by means of their DA receptor blocking properties (Bunney et al. 1973), such a mechanism can probably not account for the similar effect of ritanserin, since this drug has relatively low affinity for D_2 receptors (Leysen et al. 1985). In addition, the present study showed that a small dose of apomorphine still causes inhibition of the DA cell firing after ritanserin administration. Thus, ritanserin causes profound activation of midbrain DA cells without concomitant blockade of DA receptors. Clearly, such an action on the nigrostriatal DA system may be relevant for the purported antiparkinsonian effect of the drug. In addition, ritanserin-induced activation of the mesolimbic DA cells may serve as one underlying mechanism for the mood elevating action of the compound as well as associated enhancement of motivation and drive, since the mesolimbic DA system has been profoundly implicated both in reward mechanisms (see Bozarth 1986) and in motivational behaviour (Papp and Bal 1986).

The present results further indicate that the activation of midbrain DA cells by ritanserin is an indirect action, requiring intact stores of endogenous 5-HT, since previous depletion of 5-HT by pretreatment with PCPA abolished this action of the drug. Thus, it can be concluded that systemic ritanserin administration induces disinhibition of the DA cells, i.e., release from a tonic 5-HT_2 receptor-mediated control mechanism.

In contrast to the almost immediate, activating effect of the 5-HT_2 receptor antagonist on midbrain DA cell activity, depletion of endogenous 5-HT by repeated PCPA treatment caused but a slight and insignificant increase in firing rate (Table 1). However, repeated PCPA treatment will impair 5-HT function gradually over more than 3 days, thus allowing various compensatory mechanisms to develop. In addition, the possibility that various 5-HT receptor subtypes may differentially influence DA cell firing cannot be excluded. Thus, the consequences of acute, specific 5-HT_2 receptor antagonism and sustained 5-HT synthesis inhibition, respectively, for DA cell firing should not necessarily be the same.

In view of previous morphological and physiological evidence (cf., Introduction), the localization of the effect of ritanserin might be within the midbrain DA cell clusters. However, whereas Dray et al. (1976) found that microiontophoretic application of 5-HT causes inhibition of firing of unidentified nigral neurons, two other studies found no effect of 5-HT on pharmacologically and antidromically identified midbrain DA neurons (Aghajanian and Bunney 1974; Collingridge and Davies 1981). Thus, microiontophoretic experiments do not provide unanimous support for 5-HT-mediated inhibition of these DA cells. Yet, in a recent study 5-HT was found to specifically affect membrane currents of ZC-SN cells, but only when applied to distal dendritic fields extending into the zona reticulata (Nedergaard et al. 1988a). Moreover, this action was antagonized by cinanserin, which blocks 5-HT_2 receptors. Also, previous experiments have revealed that 5-HT can increase the release of dendritic DA within the SN, an effect which

is calcium dependent and antagonized by cinanserin (Williams and Davies 1983). In fact, the facilitatory effect of 5-HT on dendritic release of DA in the SN seems similar to that of amphetamine. Recently two reports have provided evidence that amphetamine can cause inhibition of DA cell firing locally within the SN (Nedergaard et al. 1988b) and in the VTA (Viscardi et al. 1987) by release of DA from the dendrites. Consequently, ritanserin may disinhibit DA cell firing by antagonizing 5-HT-induced release of DA and subsequent autoinhibition of the DA neurons. Nevertheless, the rather low density of 5-HT_2 receptors in the rat SN and VTA, respectively, as judged by autoradiographic evidence (Pazos et al. 1985), might suggest that ritanserin's primary site of action to disinhibit DA cell firing is localized at some other site in brain.

The significant, long lasting activation of DA cell firing by ritanserin is of considerable interest in view of its reported therapeutic action on so-called negative symptoms in chronic (type II) schizophrenia, which are relatively resistant to treatment with conventional neuroleptics. In fact, some clinical findings indicate a reduced central DA function in this degenerative type of schizophrenia (van Kammen et al. 1983; Karoum et al. 1987).

The thymostenic effects of ritanserin, i.e., mood elevation and improvement of energy have, so far, been explained by the increased slow wave sleep induced by the drug (Reyntjens et al. 1986). The present study allows the additional interpretation that such behavioural effects, as well as improvement in parkinsonism and chronic schizophrenia, may be related to activation of midbrain DA systems through release from serotonergic inhibition.

Acknowledgements. This study was supported by grants from the Swedish Medical Research Council (project no 04747), Svenska Tobaks AB (grant no 8618), The Council for Tobacco Research, USA, Inc. (grant no 2192), Torsten och Ragnar Söderbergs Stiftelser, Karolinska Institute and the Swedish Society of Medicine. Luisa Ugedo was supported by fellowships from MEC (Madrid) and Karolinska Institute. We thank Ms Monica Marcus and Ms Ulla Eriksson for skillful technical assistance and Mr. Klaus Liebold, Dr. Michael Palmer and Mr. Per-Olov Elovsson for computer programs.

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Received October 12, 1988/Final version November 10, 1988