British Journal of Pharmacology (1998) 123, 1138–1142

Effect of RX 821002 at 5-HT_{1A}-receptors in rabbit spinal cord *in vivo*

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1 The activity of RX 821002 (2-methoxy idazoxan) at 5-HT_{1A}-receptors in the spinal cord has been investigated in decerebrated, spinalized rabbits. Reflexes evoked in medial gastrocnemius motoneurones by electrical stimulation of the sural nerve were unaffected by intrathecal (i.th.) administration of RX 821002 (111 and 664 nmol cumulative, $n=7$), although the highest dose of this drug did produce a significant increase in heart rate of 28 ± 7 beats min^{-1}. Subsequent administration of the 5-HT_{1A}-receptor agonist (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) at 300 nmol, i.th., facilitated reflexes to a median of 144% of pre-drug controls, an effect that was partially reversed (to a median value of 120% of pre-drug values) by subsequent administration of the 5-HT_{1A}-receptor antagonist WAY-100635, at 185 nmol i.th.

2 In a separate set of experiments, 8-OH-DPAT was given at 30 nmol i.th. and potentiated reflexes to a median of 170% of pre-drug levels ($n=8$). Subsequent administration of RX 821002 (at a cumulative dose of 1.11 μmol, i.th., $n=5$) significantly reduced gastrocnemius responses to a median of 154% of control values.

3 After a 3 h recovery period, 8-OH-DPAT was re-administered at 30 nmol, i.th., and increased reflexes to a median value of 151% of pre-drug levels, an effect not significantly different from when it was given alone. WAY-100635 dose-dependently antagonized this effect, causing significant reductions in reflexes at a cumulative dose of 0.55 nmol, i.th., and complete reversal of the effects of 8-OH-DPAT at a cumulative dose of 5.5 nmol.

4 These data show that, at intrathecal doses up to 664 nmol, RX 821002 is devoid of agonist activity at 5-HT_{1A}-receptors. It appears to be a very weak antagonist at these sites *in vivo*, being some 2000 times less potent than WAY-100635. The inability of WAY-100635 to block completely the effects of high doses of 8-OH-DPAT has been noted previously and can be explained by non-selective actions of the agonist. However, it would appear that a 30 nmol i.th. dose of 8-OH-DPAT is selective for 5-HT_{1A} receptors in this preparation.

Keywords: 5-Hydroxytryptamine; adrenoceptors; reflex

Introduction

RX 821002 (2-methoxy-idazoxan) is a selective α_2-adrenoceptor antagonist (Welbourn *et al*., 1986; Hudson *et al*., 1992). Compared to other drugs of this type, it has the advantage of not binding to any of the known types of imidazoline receptors (Hudson *et al*., 1992; Miralles *et al*., 1993, although see Callado 1996). However, it is known to bind with moderate affinity to 5-HT_{1A}-receptors (Vauquelin *et al*., 1990; Javier Meana *et al*., 1996). The object of the present study was to investigate to what extent RX 821002 interacts with 5-HT_{1A}-receptors in rabbit spinal cord *in vivo*. In the decerebrated and spinalized rabbit, the archetypal 5-HT_{1A}-receptor agonist (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) increases the reflex responses of medial gastrocnemius motoneurones evoked by electrical stimulation of the sural nerve (Clarke *et al*., 1997). Thus, if RX 821002, like idazoxan (Llado *et al*., 1996), is an agonist at 5-HT_{1A}-receptors, it should increase reflexes in spinalized rabbits. In contrast, if it is an antagonist, RX 821002 would reduce the facilitation of reflexes by 8-OH-DPAT. The present experiments have been designed on this basis.

Methods

Experiments were performed on 17 rabbits of either sex and weighing between 1.7 and 3.0 kg. Anaesthesia was induced by i.v. injection of methohexitone sodium (4 mg kg^{-1} initially) and was continued, after cannulation of the trachea, with halothane (1.5–4%) delivered in N_2O (70%): O_2 (30%). The left carotid artery and jugular vein were cannulated for recording of arterial blood pressure and drug administration, respectively. Core temperature was maintained between 37.5 and 38.5°C by a thermostatically-controlled heating blanket. A laminectomy was performed between vertebrae L1 and L2 and a cannula was inserted beneath the dura at this point so that its tip was at or near segments L7 and S1. The animals were decerebrated by suction to the pre-collicular level after which the cranial cavity was filled with warmed paraffin oil (38°C). The left hind limb was clamped by the tibia and femur and an incision was made in the biceps femoris muscle, the parts of which were retracted to create the walls of a pool that was filled with warmed paraffin oil (38°C) after nerve dissection. The sural nerve was dissected free of connective tissue, cut near the popliteal artery and placed over platinum wire stimulating electrodes. The nerve supplying medial gastrocnemius (MG) was cut and the central end was placed over platinum recording electrodes.

Following all surgery, anaesthesia was discontinued, the animals were paralysed with pancuronium bromide (0.4 mg kg^{-1}, i.v., initially and maintained as required), and were artificially ventilated with oxygen-enriched room air to maintain P_aO_2 above 10 kPa, and at a stroke volume necessary to maintain P_aCO_2 between 3.5 and 4.5 kPa. No data were collected until at least one hour after withdrawal of halothane anaesthesia.

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Nerve stimulation and recording

Reflexes were evoked by stimulation of the sural nerve with square-wave pulses of 0.1 ms duration in blocks of eight, at a rate of 1 Hz and at a strength sufficient to recruit all myelinated axons in the nerve (20–40 times threshold). Every two minutes reflex responses to these stimuli were recorded from the MG muscle nerve, which was crushed between the electrodes to give a monophasic signal. The responses to each set of eight successive stimuli were averaged and integrated by computer. Responses were quantified as the voltage/time integral (area) of the averaged muscle nerve neurogram. Reflexes were recorded for at least thirty minutes to ensure stability of the response before the administration of any drugs.

Drugs administration

Two sets of experiments were performed. In the first, RX 821002 was given intrathecally (i.th.) before any other drugs, in doses of 30 and then 150 μg (111 and 553 nmol), followed by 8-OH-DPAT (100 μg, 300 nmol, i.th.) and then WAY-100635 (100 μg, 185 nmol, i.th.), with each injection separated by 12 min intervals. The second series of experiments were usually performed in two sections. Initially, 8-OH-DPAT was administered at 10 μg (30 nmol) i.th., followed by RX 821002 at 30, 120 and, in five animals, 150 μg i.th. to give cumulative doses of 150 or 300 μg (553 or 1108 nmol). The preparations were then allowed to recover for 3 h before 30 nmol 8-OH-DPAT (i.th.) was repeated, followed by increasing doses of WAY-100635 of 0.3, 0.7, 2, and 7 μg (0.55, 1.3, 3.7, and 13 nmol) to give a final cumulative dose of 10 μg (18.5 nmol), i.th. Two animals received only the first section of these treatment protocols and two others only the second. In both parts of the experiment, 12 min were allowed after the injection of 8-OH-DPAT and 6 min after each injection of RX 821002 or WAY-100635. Drugs were administered in volumes of 5–37.5 μl, and flushed in with 50 μl of Ringer-Dale solution. It has been shown many times that i.th. administration of similar volumes of Ringer-Dale has no effect on reflex responses in this preparation (Harris & Clarke, 1992; 1993).

Drugs

Methohexitone sodium (Brietal, Eli Lilly) was dissolved to 10 mg ml^{-1} in distilled water. All of the following drugs were dissolved in Ringer-Dale solution: pancuronium bromide (David Bull Laboratories), was diluted to 0.4 mg ml^{-1}; RX 821002.HCl (2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline), a gift of Dr D. Nutt (Psychopharmacology Unit, Bristol), was dissolved to 15 mmol l^{-1} (4 mg ml^{-1}) and diluted to 1.5 mmol l^{-1} where necessary; (±)- 8-OH-DPAT.HBr (Research Biochemicals Inc.), dissolved to a stock concentration of 6.05 mmol l^{-1} (2 mg ml^{-1}) and diluted as required; and WAY-100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide.3HCl), a gift of Wyeth Research U.K., dissolved to a strength of 3.70 mmol l^{-1} (2 mg ml^{-1}) and diluted as required. The Ringer-Dale solution used in this laboratory has the following composition: NaCl 154 mmol l^{-1}, KCl 4 mmol l^{-1}, $NaHCO_3$ 6 mmol l^{-1}, $CaCl_2$ 2.2 mmol l^{-1} and $MgCl_2$ 50 μmol l^{-1}.

Statistical analysis

Reflex responses are expressed as a percentage of the mean of the values recorded during the 24 min pre-drug control period (defined as 100%). The effects of RX821002 and WAY-100635 after 8-OH-DPAT are expressed in terms of the change in the size of reflex responses induced by the 5-HT$_{1A}$-receptor agonist, so that in this case the difference between pre- and post-8-OH-DPAT levels was taken as 100%. Reflex data were not normally distributed and were analysed by Friedman's ANOVA on ranks, Wilcoxon's matched pairs or signed ranks tests for paired data and the Mann-Whitney U-test for unpaired data. The data are presented as medians and inter-quartile ranges (IQR) from each group of experiments.

Blood pressure and heart rate data were suitable for parametric analysis and are presented as means ± s.e.mean. They were analysed by 1-way ANOVA and paired or unpaired *t* tests as appropriate. All tests were performed by use of the InStat program from GraphPad software.

Results

RX 821002 given first

RX 821002 had no effects on MG reflex responses to sural nerve stimulation (Figure 1). After the higher dose of the adrenoceptor antagonist (664 nmol cumulative), responses were a median of 99% (IQR 95–107%) of pre-drug values ($P=0.7$, Wilcoxon test, $n=7$).

Twelve minutes after RX 821002 and given at 300 nmol, i.th., 8-OH-DPAT increased the sural-MG reflex to a median value of 144% (IQR 131–216%, Figure 1) of pre-drug levels, an effect that was significant with respect to both pre-drug controls and post-RX 821002 levels (Friedman's ANOVA, $P=0.0008$, followed by Wilcoxon tests, $P=0.016$ in both cases). The increase was partially reversed by WAY-100635 (185 nmol, i.th.) given 12 min later, so that reflexes were a median value of 120% (IQR 114–176%) of pre-drug values. Although the decrease in reflexes induced by WAY-100635 was statistically significant with respect to post-8-OH-DPAT values (Wilcoxon test, $P=0.016$), reflexes after the 5-HT$_{1A}$-receptor antagonist were still significantly greater than the original pre-drug levels ($P=0.016$, Wilcoxon test).

In the control state, before the administration of RX 821002, the mean arterial blood pressure in these preparations

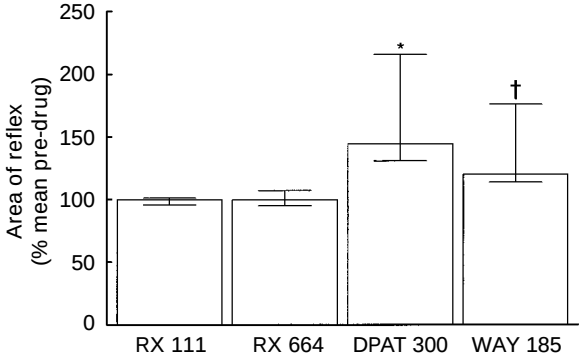

Figure 1 The effects of intrathecal RX 821002 (RX, 111 and then 664 nmol cumulative doses) on sural-MG reflexes in decerebrated, spinalized rabbits, followed by 8-OH-DPAT (DPAT) at 300 nmol and WAY-100635 (WAY) at 185 nmol i.th.. The height of each column represents the median value from 7 experiments, and the vertical lines indicate inter-quartile ranges (IQRs). Friedman's ANOVA indicated significant differences across treatments ($P=0.008$). *$P<0.02$ compared to pre-drug and post-RX821002 levels (Wilcoxon test), †$P<0.02$ compared to post-8-OH-DPAT, post-RX 821002 and pre-drug values (Wilcoxon test).

was 61±4 mmHg and heart rate was 273±9 beats min^{-1}. No significant changes in blood pressure were observed (ANOVA, $P=0.34$ across all treatments), but a significant increase in heart rate occurred after the higher dose of RX 821002 (mean change from control 28±7 beats min^{-1}, ANOVA $P=0.001$, followed by Student-Newman-Keuls test, $P<0.01$). 8-OH-DPAT reduced heart rate so that it was no longer significantly different from pre-drug control, but this effect was completely reversed by WAY-100635 and heart rate was restored to its post-RX 821002 level, becoming significantly higher than in the pre-drug control period (Figure 2).

8-OH-DPAT given first

8-OH-DPAT and subsequent administration of RX 821002 Given intrathecally at 30 nmol, 8-OH-DPAT increased MG reflexes significantly, to a median value of 170% (IQR 148–227%) of pre-drug values (Wilcoxon test, $P=0.008$, $n=8$). Neither the 111 nor the 553 nmol cumulative doses of RX 821002 had any significant effect on reflexes previously facilitated by 8-OH-DPAT, although the higher dose did decrease reflexes in 6 of 8 animals tested (Wilcoxon tests, $P=0.9$ and 0.19 for the two doses, respectively Figure 3). Five animals received the highest dose of RX 821002 (1.11 μmol, i.th., cumulative). Analysing the results from these preparations only, the highest dose of RX 821002 reduced reflexes significantly (Friedman's ANOVA, $P=0.018$, followed by Dunn's test, $P<0.05$), so that 8-OH-DPAT-induced facilitation was reduced to a median of 71% (IQR 48–75%) of its original value.

In these experiments mean arterial pressure before drugs was 70±2 mmHg and heart rate was 287±9 beats min^{-1}. Heart rate decreased by 7±3 beats min^{-1} with 8-OH-DPAT, and increased by 14±7 and 19±9 beats min^{-1} with the two higher doses of RX 821002, respectively, but these changes were not statistically significant (ANOVA, $P=0.08$). Mean arterial pressure did not change significantly in these experiments (ANOVA, $P=0.3$).

8-OH-DPAT and subsequent administration of WAY-100635 Reflexes tended to be larger after the 3 h rest (median 140 μV ms, IQR 103–151) than in the original control period (median 78 μV ms, IQR 50–103), so new baselines were established for this part of the experiment. The second administration of 8-OH-DPAT at 30 nmol i.th. caused reflexes to increase to a median of 151% (IQR 139–188%) of pre-8-OH-DPAT levels. The increase induced by this dose was not significantly different from that seen on the first application (Mann-Whitney U test, $P=0.33$). Subsequent administration of WAY-100635 caused a dose-dependent reduction in reflexes towards pre-8-OH-DPAT levels (Figure 3), that was highly statistically significant (Friedman's ANOVA, $P=0.0001$) and that became so at the lowest dose of the antagonist used (0.55 nmol, Wilcoxon test, $P=0.04$). The effects of 8-OH-DPAT were completely reversed with a cumulative i.th. dose of 5.5 nmol WAY-100635 (Figure 3), at which point reflexes were 95% (IQR 89–103%) of pre-8-OH-DPAT levels. The reduction in 8-OH-DPAT-induced facilitation by RX 821002 (1.11 μmol cumulative) was not significantly different from that seen with 0.55 nmol WAY-100635, but was significantly less than that achieved with a 1.85 nmol cumulative dose of the 5-HT$_{1A}$-receptor antagonist (Mann-Whitney tests, $P=0.9$ and 0.04 respectively). For this part of the study, mean arterial pressure before 8-OH-DPAT was 66±4 mmHg and heart rate was 296±9 beats min^{-1}. The combination of drugs used in this part of the study had no significant effects on cardiovascular parameters (ANOVA, $P=0.7$ and 0.13 for arterial pressure and heart rate, respectively). The two animals in which this was the only experiment performed showed the same responses to 8-OH-DPAT and WAY-100635 as those receiving two doses of 8-OH-DPAT.

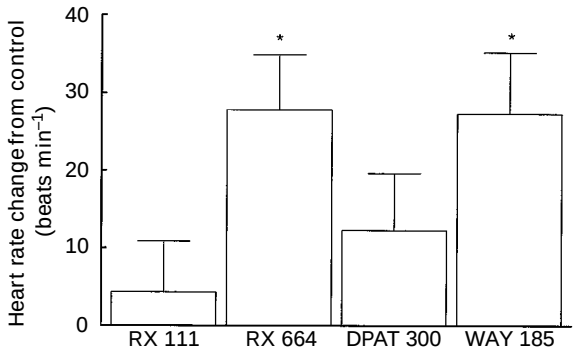

Figure 2 The effects of intrathecal RX 821002 (RX, 111 and then 664 nmol cumulative doses) on heart rate in decerebrated, spinalized rabbits, followed by 8-OH-DPAT (DPAT) at 300 nmol and WAY-100635 (WAY) at 185 nmol, i.th. The height of each column represents the mean value from 7 experiments, and the vertical lines indicate s.e.means. ANOVA for repeated measures indicates significant differences across these treatments ($P=0.001$). *Significantly greater than pre-drug controls.

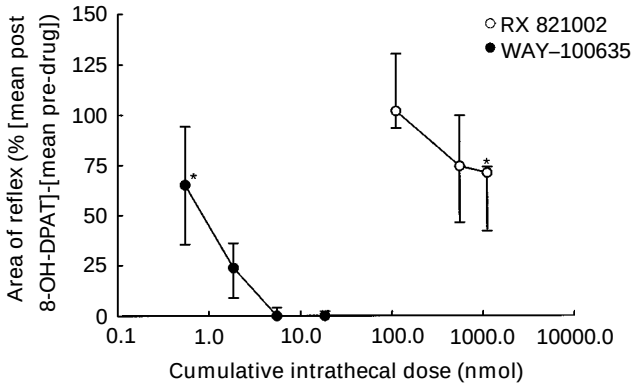

Figure 3 Dose-response curves showing effects of intrathecal WAY-100635 and RX 821002 on sural-MG reflexes facilitated by 8-OH-DPAT at 30 nmol, i.th. In this graph 100% represents the difference between the control and post-8-OH-DPAT values. Each point (except for the 1.11 μmol dose of RX 821002 where $n=5$) is the median from 8 experiments, and the vertical lines indicate IQRs. *Indicates lowest dose causing a statistically significant decrease from post 8-OH-DPAT levels.

Discussion

Previous work from this laboratory has shown that idazoxan, RX 821002 and RX 811059 (2-ethoxy-idazoxan) increase MG reflex responses in non-spinalized, decerebrated rabbits, an effect that we had ascribed to blockade of inhibitory spinal α_2-adrenoceptors (Harris & Clarke, 1992; 1993). Maximum facilitation of reflexes was seen with cumulative i.th. doses of RX 821002 between 55 and 240 nmol and an approximate ED_{50} of 37 nmol (Harris & Clarke, 1993). More recently we have shown that the selective 5-HT$_{1A}$-receptor antagonists WAY-100135 and WAY-100635 also increase the sural-MG reflex in rabbits (Clarke *et al.*, 1996), with the same maximum

effect as RX 821002 but with an ED_{50} in the case of the latter agent of only 0.9 nmol. It was therefore very important for us to assess the *in vivo* potency of RX 821002 as a 5-HT$_{1A}$-receptor ligand to establish to what extent, if any, our previous results with this drug could be ascribed to α_2- or 5-HT$_{1A}$-receptors.

RX 821002 has been shown to displace 8-OH-DPAT from rat cortical membranes with an IC_{50} of around 30 nmol l^{-1} (Vauquelin *et al*., 1990), and its progenitor drug, idazoxan, has been found to have partial agonist activity at these receptors *in vitro* (Llado *et al*., 1996). On the basis of these data, one would predict that RX 821002 could act as either a reasonably potent antagonist or possibly even an agonist at 5-HT$_{1A}$-receptors. 8-OH-DPAT, often described as a selective 5-HT$_{1A}$-agonist, increases MG reflex responses to sural nerve stimulation in spinalized rabbits (Clarke *et al*., 1997). In the present study (also in spinalized preparations), RX 821002 had no effect on sural-MG reflexes at a dose of up to 664 nmol, i.th. We may conclude then that RX 821002 is devoid of detectable agonist activity at 5-HT$_{1A}$-receptors up to this dose level. Furthermore, the results of the present experiments confirm that the large increases in reflexes and arterial blood pressure induced by i.th. RX 821002 in non-spinalized animals (Harris & Clarke, 1993) is the result of blockade of descending systems acting in the spinal cord, rather than due to leakage of the drug into the blood stream. On the other hand, the increase in heart rate resulting from RX 821002 administration in the present study presumably did come from leakage into the circulation. The subsequent changes in heart rate induced by 8-OH-DPAT and WAY-100635 are quite consistent with the previously demonstrated bradycardic action of the 5-HT$_{1A}$-agonist and its reversal by the antagonist (see Clarke *et al*., 1997). These findings show that these agents are not confined to the intrathecal space when injected via that route. Presumably the failure of RX 821002 significantly to increase heart rate in the presence of 8-OH-DPAT is due to the bradycardic actions of the former drug.

In the presence of RX 821002, 8-OH-DPAT at 300 nmol (i.th.) increased reflexes in the same way that it does when given i.v. (Clarke *et al*., 1997), and this effect was partially but not completely reversed by WAY-100635, at 185 nmol (i.th.). These observations show that RX 821002 had not blocked a significant number of 5-HT$_{1A}$-receptors at the doses used. The inability of WAY-100635 to reverse fully the effects of this large dose of 8-OH-DPAT is the result of the agonist interacting with other 5-HT receptors (Clarke *et al*., 1997).

Having shown that RX 821002 did not block the effects of a large dose of 8-OH-DPAT, it was decided to investigate its potential antagonist activity against a lower dose of the agonist. At 30 nmol (i.th.) 8-OH-DPAT induced reliable increases in the sural-MG reflex of the same magnitude as seen with ten times the dose, a result consistent with the flat dose-response relationship obtained for this drug when it was given i.v. (Clarke *et al*., 1997). A very large dose of RX 821002 (1.11 μmol cumulative) resulted in partial reversal of the facilitatory effects of the lower dose of 8-OH-DPAT, having an equivalent effect to 0.55 nmol of the selective 5-HT$_{1A}$-receptor antagonist WAY-100635 (Fletcher *et al*., 1996). The most parsimonious explanation of this result is that RX 821002 is a very weak antagonist at 5-HT$_{1A}$-receptors *in vivo*, with a potency some 2000 times less than WAY-100635. A further, and very useful, observation to come from this study is that WAY-100635 completely reversed the effects of 8-OH-DPAT at 30 nmol i.th., showing that this dose of the agonist is selective for 5-HT$_{1A}$-receptors. Our data indicate that a molar dose-ratio of 1:5 is sufficient for WAY-100635 to abolish completely the agonist activity of 8-OH-DPAT in this preparation. Both RX 821002 and WAY-100635 increase MG reflex responses to sural nerve stimulation in decerebrated, non-spinalized rabbits (Harris & Clarke, 1993; Clarke *et al*., 1996), whereas neither drug has any significant actions in spinalized preparations. We have taken this to mean that the sural-MG reflex pathway is under tonic inhibition emanating from adrenergic and 5-hydroxytryptaminergic neurones in the brain stem and mediated through spinally located α_2-adrenoceptors and 5-HT$_{1A}$-receptors, respectively. Although WAY-100635 was some 40 times more potent than RX 821002 in increasing reflexes (see above), this difference is too small to be accounted for by an antagonist effect of RX 821002 at 5-HT$_{1A}$-receptors. Presumably the difference between the two drugs arises in part from the slightly lower potency of RX 821002 at α_2-adrenoceptors (K_D values for rabbit of about 6 nmol l^{-1}, Trendelenburg *et al*., 1996) compared to WAY-100635 at 5-HT$_{1A}$-receptors (K_D about 1 nmol l^{-1}, Fletcher *et al*., 1996). It may also indicate that there is more noradrenaline to displace from inhibitory receptors than 5-HT, or that the adrenoceptors involved are located in deeper tissues and less accessible to drugs given by the i.th. route (Bras *et al*., 1990). Furthermore, RX 821002 in non-spinalized rabbits produced large increases in blood pressure (of the order of 25 mmHg), whereas WAY-100635 had no effect at all on cardiovascular variables. Taking into account all relevant observations, we are confident that the effects of RX 821002 observed in previous studies are due to blockade of α_2-adrenoceptors rather than 5-HT$_{1A}$-receptors.

This work was supported by the Wellcome Trust. Our thanks to Professor David Nutt (University of Bristol) and to Wyeth U.K. (as was) for the supply of RX 821002 and WAY-100635, respectively. J.O. is a University of Nottingham scholar.

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(Received September 26, 1997
Revised December 8, 1997
Accepted December 9, 1997)