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Modulation of NMDA effects on agonist-stimulated phosphoinositide turnover by memantine in neonatal rat cerebral cortex

Rajendra Mistry, *Rolf Wilke & [1]R.A. John Challiss

Department of Cell Physiology and Pharmacology, University of Leicester, P.O. Box 138, Medical Sciences Building, University Road, Leicester LE1 9HN and *Merz & Co., Eckenheimer Landstrasse 100-104, 60318 Frankfurt am Main, Germany

1 The ability of memantine (1-amino-3,5-dimethyladamantane) to antagonize the modulatory effects of N-methyl-D-aspartate (NMDA) on phosphoinositide turnover stimulated by muscarinic cholinoceptor- and metabotropic glutamate receptor-agonists has been examined in neonatal rat cerebral cortex slices.

2 Memantine antagonized the inhibitory effect of NMDA (100 μM) on both total [^{3}H]-inositol phosphate ([^{3}H]-InsP_x) and inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3) mass accumulations stimulated by carbachol (1 mM) with EC_{50} values of 21 and 16 μM respectively.

3 Memantine concentration-dependently antagonized (IC_{50} 24 μM) the ability of NMDA (10 μM) to potentiate [^{3}H]-InsP_x accumulation in response to a sub-maximal concentration of the metabotropic glutamate receptor agonist, 1S,3R-ACPD (10 μM).

4 The small (approx. 3 fold), concentration-dependent increase in [^{3}H]-InsP_x accumulation stimulated by NMDA was completely antagonized by the prototypic NDMA receptor-channel blocker, MK-801 (1 μM) at all concentrations of NDMA studied (1-1000 μM). In contrast, antagonism by memantine (100 μM) was observed only at low concentrations of NMDA (1-10 μM), whilst [^{3}H]-InsP_x accumulation stimulated by high concentrations of NMDA (300-1000 μM) was markedly enhanced by memantine.

5 Assessment of the incorporation of [^{3}H]-inositol into inositol phospholipids revealed that memantine (100 μM) caused an approximate 2 fold increase in the labelling of phosphatidylinositol, phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate.

6 H.p.l.c. separation of [^{3}H]-inositol (poly)phosphates demonstrated that whilst memantine (100 μM) alone had no significant effect on the accumulation of any isomer, it substantially altered the profile of accumulation stimulated by NMDA (1 mM), greatly facilitating accumulation of Ins(1,4,5)P_3 and inositol 1,3,4,5-tetrakisphosphate (Ins(1,3,4,5)P_4).

7 These data provide evidence that memantine can antagonize the actions of NMDA in neonatal rat cerebral cortex slices in a manner consistent with this agent acting as a NMDA receptor-channel blocker. In addition, at least two further actions of memantine can be proposed. Memantine increases the rate of [^{3}H]-inositol incorporation into the cellular inositol phospholipid fraction, without significantly stimulating phosphoinositide turnover. Furthermore, memantine can substantially alter patterns of inositol (poly)phosphates stimulated by NMDA, promoting the accumulation of the established and putative second messengers Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 which are not increased by NMDA in the absence of memantine. It is unknown whether these latter loci of memantine action contribute to known therapeutic actions of this agent.

Keywords: NMDA-receptor; memantine (1-amino-3,5-dimethyladamantane); metabotropic glutamate receptor; muscarinic cholinoceptor; phosphoinositide turnover; inositol 1,4,5-trisphosphate; cerebral cortex (neonatal rat)

Introduction

Memantine (1-amino-3,5-dimethyladamantane) is used clinically in the treatment of Parkinson's disease and neurogenic motor disorders such as spasticity (Grossman & Schutz, 1982; Wesemann *et al.*, 1983; Schneider *et al.*, 1984). Evidence is also accruing to suggest that memantine may be a useful therapeutic agent in cerebrovascular diseases and dementia-related cognitive deficits (Wesemann *et al.*, 1983; Ditzler, 1991; Goertelmeyer & Erbler, 1992).

Although initial data pointed to memantine being an 'enhancer' of dopaminergic transmission (Maj *et al.*, 1974), recent work has demonstrated that memantine displaces [^{3}H]-MK-801 binding (Kornhuber *et al.*, 1989), blocks NMDA-receptor activation (Bormann, 1989) and antagonizes the consequences of NMDA-receptor activation (Erdö & Schaefer, 1991; Keilhoff & Wolf, 1992; Lupp *et al.*, 1992). These findings have been confirmed by patch clamp methodologies which have shown that memantine is an uncompetitive open channel blocker of the NMDA-receptor cation channel, effective at low micromolar concentrations and exhibiting weaker use-dependency and stronger voltage-dependency compared to MK-801 (Chen *et al.*, 1992; Parsons *et al.*, 1993).

Memantine has also been reported to exert a number of effects in neuronal preparations which are unlikely to be a consequence of NMDA-receptor blockade. In particular, memantine has been reported to stimulate inositol phosphate accumulation in retinal cell cultures and slices prepared from a number of brain regions (Osborne & Quack, 1992).

We have recently investigated the complex modulatory actions of NMDA on muscarinic cholinoceptor- and metabotropic glutamate receptor-stimulated phosphoinositide turnover in neonatal rat cerebral cortex (Challiss *et al.*, 1994a,b). In the present study we have assessed under what conditions memantine antagonizes NMDA modulatory effects on agonist-stimulated phosphoinositide turnover and whether memantine alone can elicit this second messenger response. Our data provide evidence that memantine can

[1] Author for correspondence.

antagonize the actions of NMDA in this preparation and also demonstrate that memantine can affect phosphoinositide turnover by apparently novel mechanisms.

Methods

Incubation methods and extract preparation

Cerebral cortex slices from 7–8 day old neonatal rats (Wistar strain, either sex) were prepared and incubated as previously described (Challiss *et al.*, 1994a,b). Where inositol phospholipids were labelled with [^{3}H]-inositol, slices (25 µl gravity-packed) were incubated in 250 µl KHB, containing 0.5 µCi [^{3}H]-inositol, at 37°C for a period of 60 min. At the end of this period, LiCl (5 mM final concentration) was added. For inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3) and phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) mass studies, the labelling period and LiCl addition were omitted from the protocol. In all cases, incubations were terminated by addition of 300 µl ice-cold 1 M trichloroacetic acid (TCA) and immediately transferred to an ice-bath. Samples were neutralized for subsequent analysis as described previously (Challiss *et al.*, 1994a,b).

[^{3}H]-InsP_x measurement and separation of [^{3}H]-inositol (poly)phosphate isomers

The total [^{3}H]-inositol phosphate fraction ([^{3}H]-InsP_x) was recovered and quantified as reported previously (Challiss *et al.*, 1994a,b). Alternatively the total [^{3}H]-InsP_x fraction, from each experimental condition performed in triplicate, was pooled and injected onto a Partisil (10 µm) SAX analytical high performance liquid chromatography (h.p.l.c.) column equipped with a pre-column packed with Whatman pellicular anion-exchange resin. The column was washed to remove [^{3}H]-inositol and gradient elution of [^{3}H]-inositol (poly)-phosphate isomers was performed as described previously (Batty *et al.*, 1989).

Recovery and separation of [^{3}H]-inositol phospholipids

Slice pellets were sequentially washed with 5% TCA/1 mM EDTA and H_2O before phospholipid extraction as described by Downes & Wusteman (1983) and determination of either total [^{3}H]-inositol phospholipids ([^{3}H]-PtdIns(P_x)) in the chloroform phase, or deacylation and separation of [^{3}H]-glycerophosphoinositol (phosphates) ([^{3}H]-GroPIns/[^{3}H]-GroPIns(4)P/[^{3}H]-GroPIns(4,5)P_2) using Dowex-1 (formate-form) columns as previously described (Simpson *et al.*, 1987). In other experiments slice pellets were washed with 2 ml 0.9% NaCl and digested in 0.5 M NaOH for subsequent protein determination by the Lowry method.

Measurement of Ins(1,4,5)P_3 and PtdIns(4,5)P_2 mass

Mass assay of Ins(1,4,5)P_3 was performed as described previously (Challiss *et al.*, 1988). In parallel, the total phospholipid extract was processed as described previously (Challiss & Nahorski, 1993) with the Ins(1,4,5)P_3, released by alkaline hydrolysis of PtdIns(4,5)P_2, determined by mass assay.

Materials

Myo-[2-^{3}H]-inositol (17–20 Ci mmol^{-1}) and [^{3}H]-inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3; 17–20 Ci mmol^{-1}) were obtained from DuPont NEN (DuPont U.K. Ltd., Stevenage, Herts., U.K.). D-Ins(1,4,5)P_3 was purchased from Rhode Island University (Kingston, Rhode Island, U.S.A.). 1-Aminocyclopentane-1S,3R-dicarboxylic acid (ACPD) was obtained from Tocris Neuramin (Langford, Bristol, U.K.). N-methyl-D-aspartate (NMDA) and Dowex AG 1-X8 (100–200 mesh, Cl^- form) were from Sigma Chemical Co. Ltd. (Poole, Dorset, U.K.). Dowex AG 1-X8 (200–400 mesh, formate form) was obtained from Bio-Rad Laboratories (Richmond, CA, U.S.A.). MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]-cyclohepten-5,10-imine maleate) was a gift from Merck Sharp & Dohme Laboratories (Harlow, Essex, U.K.). Memantine (1-amino-3,5-dimethyladamantane) was supplied by Merz & Co. (Frankfurt am Main, Germany).

Data analysis

All values are presented as means ± s.e.mean for the indicated number of separate experiments. A computer programme (GraphPad, ISI) was used to generate EC_{50}/IC_{50} values from concentration-response data; EC_{50}/IC_{50} values are given as means with 95% confidence limits in parentheses. Statistical comparisons were performed by Student's *t* test for unpaired observations.

Results

Effects of memantine on NMDA modulation of carbachol-stimulated phosphoinositide turnover

We have previously demonstrated that NMDA has a complex modulatory effect upon carbachol-stimulated [^{3}H]-InsP_x accumulation in neonatal cerebral cortex slices (Challiss *et al.*, 1994a). Data entirely consistent with previous observations are shown in Figure 1. Exposure of slices to 1 mM carbachol for 15 min in the presence of 5 mM LiCl resulted in a 36 fold increase in [^{3}H]-InsP_x accumulation: co-addition of 10 µM NMDA caused a small (18%), but significant ($P<0.05$; unpaired Student's *t* test) increase in the agonist-stimulated response, whilst 100 µM NMDA profoundly inhibited the response (by 71%; $P<0.001$).

Neither memantine (100 µM) nor MK-801 (1 µM) affected carbachol-stimulated [^{3}H]-InsP_x accumulation, although memantine caused a small, but significant (32%; $P<0.05$) increase in basal [^{3}H]-InsP_x accumulation (Figure 1). The inhibitory effect of 100 µM NMDA on carbachol-stimulated [^{3}H]-InsP_x accumulation was completely prevented by memantine and MK-801 suggesting that like MK-801, memantine also antagonizes the effects of NMDA. However, whilst MK-801 significantly attenuated the enhancement of the agonist-stimulated [^{3}H]-InsP_x accumulation by 10 µM NMDA, memantine was without effect.

This effect of memantine was also seen with respect to carbachol-stimulated Ins(1,4,5)P_3 mass accumulation in neonatal cerebral cortex slices (Figure 2). Addition of carbachol (1 mM) caused a prompt increase in Ins(1,4,5)P_3 accumulation which reached about 6 fold over basal levels 15 s after agonist challenge and subsequently decreased to an elevated plateau level. In agreement with previous work (Challiss *et al.*, 1994a), the presence of 100 µM NMDA significantly enhanced the initial carbachol-stimulated increase in Ins(1,4,5)P_3 accumulation (by 33%; $P<0.02$), whilst with increasing time of exposure the presence of NMDA significantly suppressed agonist-stimulated Ins(1,4,5)P_3 accumulation 5 and 10 min after challenge (Figure 2). Pre-addition of 100 µM memantine had no effect on the initial enhancement of carbachol + NMDA-stimulated Ins(1,4,5)P_3 accumulation, but completely prevented the time-dependent development of the inhibitory phase. Indeed, 5 and 10 min after carbachol + NMDA addition, memantine appeared to enhance significantly the Ins(1,4,5)P_3 accumulation compared to that observed in the presence of carbachol only. These data suggest that memantine blocks the NMDA effect by 90–95%, reducing the effective NMDA concentration to a range over which only a facilitatory effect of NMDA is observed (see Discussion).

The effects of increasing concentrations of NMDA on basal and 10 min carbachol-stimulated Ins(1,4,5)P_3 accumulations were established in the absence and presence of 100 μM memantine (Figure 3). Low concentrations of NMDA significantly enhanced the carbachol-stimulated Ins(1,4,5)P_3 response (by 43% ($P<0.05$) and 78% ($P<0.001$) at 3 and 10 μM NMDA respectively), whilst high concentrations (100–1000 μM NMDA) profoundly inhibited both the basal and agonist-stimulated accumulations of Ins(1,4,5)P_3. In the presence of 100 μM memantine the bi-modal modulatory effect of NMDA was essentially lost. Thus, memantine prevented the inhibitory effect of high concentrations of NMDA on both basal and agonist-stimulated responses and appeared to shift the enhancement of carbachol-stimulated Ins(1,4,5)P_3 accumulations to higher concentrations of NMDA.

The ability of memantine to reverse the inhibitory action of NMDA (100 μM) on carbachol-stimulated [^{3}H]-InsP_x and Ins(1,4,5)P_3 accumulation is shown in Figure 4. Memantine concentration-dependently and fully reversed the inhibitory effect of NMDA; the concentration of memantine calculated to cause 50% reversals of the NMDA effect on carbachol-stimulated [^{3}H]-InsP_x and Ins(1,4,5)P_3 accumulations were respectively observed as 21.2 (95% confidence limits 17.7–25.3) and 16.0 (14.3–17.8) μM.

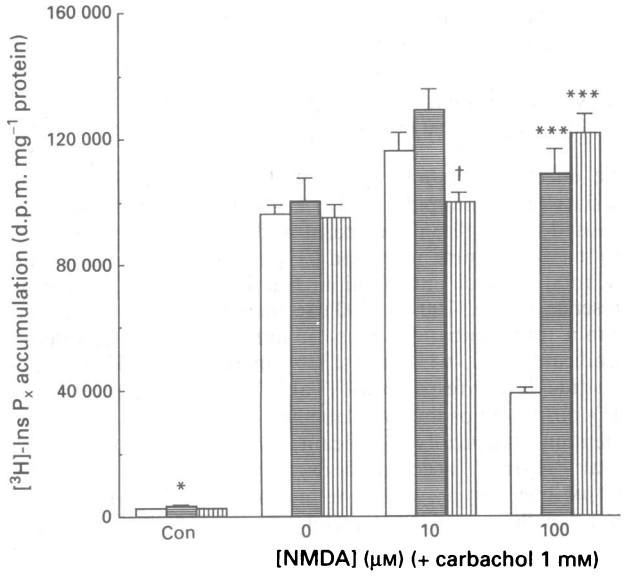

Figure 1 Effects of memantine and MK-801 on the modulation of carbachol-stimulated [^{3}H]-InsP_x accumulation by N-methyl-D-aspartate (NMDA). Either 100 μM memantine (horizontally-hatched columns), 1 μM MK-801 (vertically lined columns), or vehicle (open columns) was added 15 min prior to simultaneous addition of 1 mM carbachol and 0, 10 or 100 μM NMDA. No further agent additions are designated as Con. Values are presented as means ± s.e.mean for 4 separate experiments performed in triplicate. Statistically significant effects of memantine or MK-801 pretreatment on the subsequent response are indicated as $*P<0.05$ and $***P<0.001$ for increases over respective values and $\dagger P<0.05$ for decreases below respective control values.

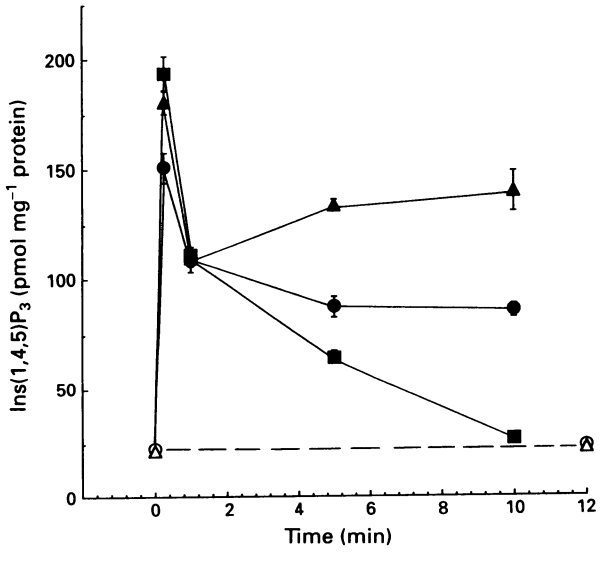

Figure 2 Effect of memantine on the time-dependent modulations of carbachol-stimulated Ins(1,4,5)P_3 mass accumulation by N-methyl-D-aspartate (NMDA). Additions of 100 μM memantine (△, ▲) or vehicle (○, ●, ■) were made 15 min before addition of 1 mM carbachol in the absence (●) or presence (■, ▲) of 100 μM NMDA. Values are presented as means ± s.e.mean for 4 separate experiments performed in triplicate.

Effects of memantine on NMDA modulation of 1S,3R-ACPD-stimulated phosphoinositide turnover

In agreement with a previous study (Challiss *et al.*, 1994b), data in Figure 5 illustrate that addition of either 10 μM 1S,3R-ACPD or 10 μM NMDA (for 15 min in the presence of 5 mM LiCl) caused only modest increases in [^{3}H]-InsP_x accumulation over basal levels (control 3323 ± 317; +10 μM 1S,3R-ACPD 13394 ± 762; +10 μM NMDA 9015 ± 887 d.p.m. mg^{-1} protein), however, co-addition of 1S,3R-ACPD + NMDA greatly potentiated the observed response (to 67267 ± 3578 d.p.m. mg^{-1} protein). Figure 5 also

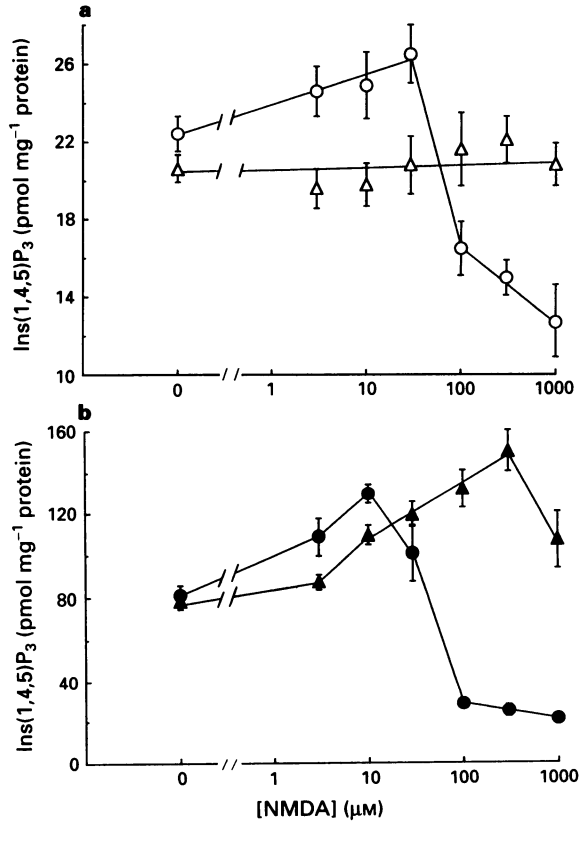

Figure 3 Effects of memantine on N-methyl-D-aspartate (NMDA) modulation of basal (a) and carbachol-stimulated (b) Ins(1,4,5)P_3 mass accumulation. Where indicated, additions of 100 μM memantine (△, ▲) or vehicle (○, ●) were made 15 min prior to addition of the indicated concentrations of NMDA in the absence (a) or presence (b) of 1 mM carbachol. Values are presented as means ± s.e.mean for 3 separate experiments performed in triplicate. Note the difference in ordinate scales between (a) and (b).

illustrates the abilities of memantine and MK-801 to antagonize the NMDA-mediated potentiation of 1S,3R-ACPD-stimulated [^{3}H]-InsP$_x$ accumulation. MK-801 fully reversed the NMDA potentiation with a 50% effect being observed at 68.1 (53.9–86.0) nM; memantine only appeared to cause an 80% reversal of the NMDA potentiation, with a half-maximal effect being observed at 24.1 (22.4–26.0) μM.

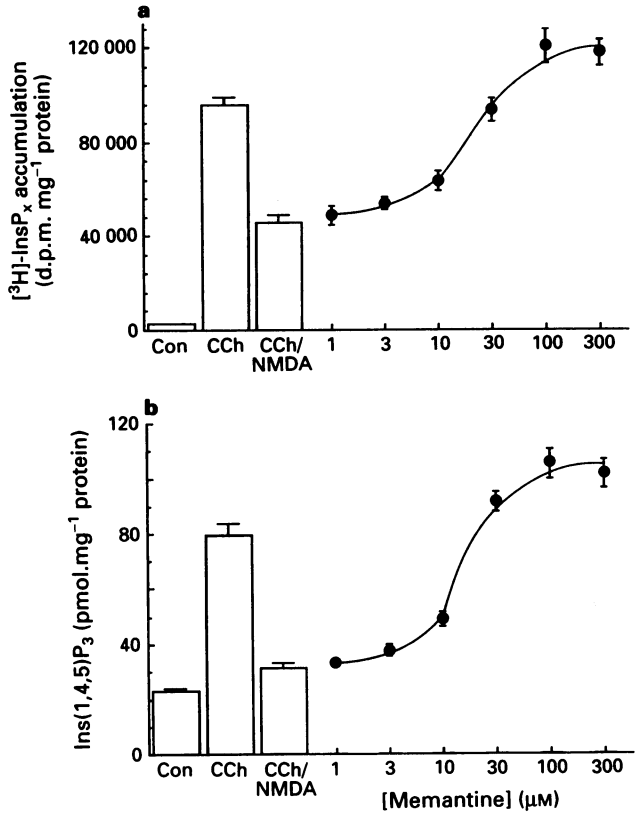

Figure 4 Concentration-dependent reversals of N-methyl-D-aspartate (NMDA)-inhibition of carbachol-stimulated [^{3}H]-InsP$_x$ (a) and Ins(1,4,5)P$_3$ (b) accumulations. The indicated concentrations of memantine were added 15 min prior to addition of 1 mM carbachol + 100 μM NMDA. The histograms on the left of each panel show the effects of vehicle (Con), 1 mM carbachol (CCh) and 1 mM carbachol + 100 μM NMDA (CCh/NMDA). Values are presented as means ± s.e.mean for 3 separate experiments performed in triplicate.

These studies all point towards memantine exerting an effect similar to MK-801, albeit with a 300–400 fold lower potency. However, memantine appears to possess properties distinct from MK-801. As reported above, NMDA caused a small concentration-dependent accumulation of [^{3}H]-InsP$_x$ (Figure 6), which was largely antagonized by MK-801 (1 μM). In contrast, whilst memantine (100 μM) appeared to antagonize [^{3}H]-InsP$_x$ accumulations stimulated by low concentrations of NMDA, it potentiated the response observed at high concentrations of NMDA (Figure 6).

Does memantine affect phosphoinositide turnover independently of NMDA-receptor antagonism?

The effects of memantine on [^{3}H]-inositol labelling of the inositol phospholipids and [^{3}H]-InsP$_x$ accumulation were assessed in parallel under basal and NMDA-stimulated conditions. Memantine increased [^{3}H]-inositol incorporation into the total inositol phospholipid fraction (Table 1). Thus, the presence of 30, 100 or 300 μM memantine for 30 min (i.e. 15 min pre-incubation plus 15 min ± NMDA) resulted in significant 30%, 64% and 137% increases in [^{3}H]-phosphoinositide levels respectively (Table 1). The enhancement of [^{3}H]-inositol phospholipid labelling was reflected to a lesser extent in statistically significant 25% and 84% increases in basal [^{3}H]-InsP$_x$ accumulations in the presence of 100 and 300 μM memantine, whilst the memantine-evoked increases in lipid labelling could completely account for the respective observed increases in NMDA-stimulated [^{3}H]-InsP$_x$ accumulation (Table 1).

The enhancement of [^{3}H]-inositol phospholipid labelling by memantine appeared to be independent of the ability of this agent to antagonize NMDA-receptor activation. Thus, pre-incubation with MK-801 inhibited NMDA-stimulated [^{3}H]-InsP$_x$ accumulation by 65% in the absence, and by 69% in the presence of 100 μM memantine, whilst having no effect on [^{3}H]-inositol phospholipid labelling. The time-course of the enhancement of [^{3}H]-inositol incorporation into the phospholipid fraction evoked by 100 μM memantine is shown in Figure 7. In this series of experiments memantine was added 30 min after initiation of labelling to allow the effects of this agent to be followed over a longer (90 min) time-course. Under basal conditions a linear rate of [^{3}H]-inositol labelling of phospholipids was observed over the 120 min time course. MK-801 (1 μM) had no effect on the rate of phospholipid

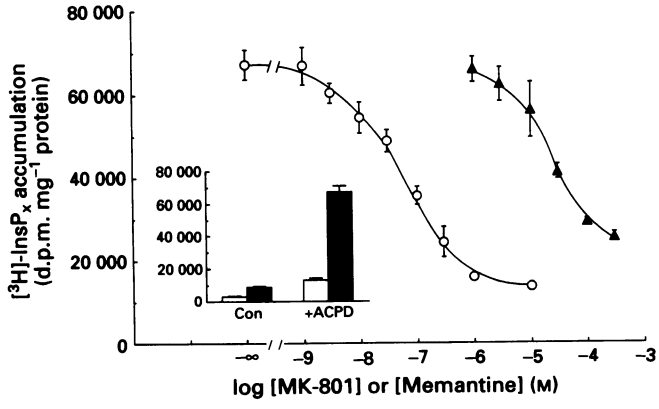

Figure 5 Concentration-dependent inhibitions of N-methyl-D-aspartate (NMDA)-potentiated 1-aminocyclopentane-1S,3R-dicarboxylic acid (1S,3R-ACPD)-stimulated [^{3}H]-InsP$_x$ accumulation by memantine and MK-801. The indicated concentrations of memantine (▲) or MK-801 (○) were added 15 min prior to simultaneous addition of 10 μM 1S,3R-ACPD and 10 μM NMDA. The inset shows the basal (Con) and 10 μM 1S,3R-ACPD-stimulated (ACPD) [^{3}H]-InsP$_x$ accumulation in the absence (open columns) or presence (solid columns) of 10 μM NMDA. In all cases values are presented as means ± s.e.mean for 3 separate experiments performed in triplicate.

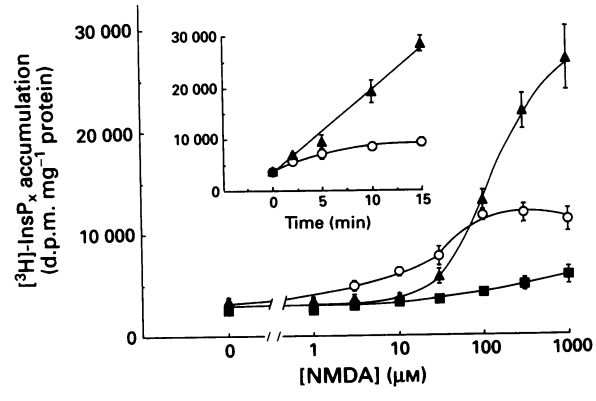

Figure 6 Effect of memantine and MK-801 on N-methyl-D-aspartate (NMDA)-stimulated [^{3}H]-InsP$_x$ accumulation. Memantine (100 μM; ▲), MK-801 (1 μM; ■) or vehicle (○) was added 15 min prior to addition of the indicated concentrations of NMDA. The inset shows the time-courses of [^{3}H]-InsP$_x$ accumulation stimulated by 1 mM NMDA in the absence (○) or presence (▲) of 100 μM memantine. In all cases, values are presented as means ± s.e.mean of 3 separate experiments performed in triplicate (main figure), or one typical experiment performed in triplicate (inset figure).

Table 1 Effects of memantine on total [^{3}H]-phosphoinositide labelling and [^{3}H]-InsP$_x$ accumulation under basal and N-methyl-D-aspartate (NMDA)-stimulated conditions

± NMDA (1 mM)	Memantine (μM)	[^{3}H]-PtdIns(P$_x$) (d.p.m. per 25 μl slices)	[^{3}H]-InsP$_x$	Ratio
−	0	76656 ± 1135	3647 ± 164	0.048
−	10	80792 ± 2981	3699 ± 341	0.046
−	30	99576 ± 4171[a]	4211 ± 511	0.042
−	100	125917 ± 3241[b]	4553 ± 241[a]	0.036
−	300	181603 ± 1873[b]	6695 ± 306[b]	0.037
+	0	65728 ± 3339[c]	10764 ± 106[d]	0.164
+	10	68978 ± 3651[c]	11039 ± 532[d]	0.160
+	30	87915 ± 2231[a]	14284 ± 181[a,d]	0.162
+	100	120728 ± 4068[b]	21094 ± 498[b,d]	0.175
+	300	155563 ± 6528[b,c]	28661 ± 1815[b,d]	0.184
+*	0	69879 ± 2292	6102 ± 833[e]	0.087
+*	100	118271 ± 2106[b]	9632 ± 641[f]	0.073

Where indicated *MK-801 (1 μM) was added 5 min prior to memantine (and 20 min prior to NMDA) addition. Total inositol phospholipid ([^{3}H]-PtdIns(P$_x$)) and inositol phosphate ([^{3}H]-InsP$_x$) fractions were recovered as described in the Methods section. Data are presented as means ± s.e.mean for a single experiment performed in quadruplicate. Essentially similar data were obtained in one further experiment. The final column gives the ratio of [^{3}H]-InsP$_x$ to [^{3}H]-PtdIns(P$_x$) under each condition. Statistically significant differences are indicated as [a]$P<0.05$, [b]$P<0.001$ increases over respective control (−memantine) values; [c]$P<0.05$ decrease in presence of NMDA compared to respective −NMDA value; [d]$P<0.001$ increase in the presence of NMDA compared to respective −NMDA value; [e]$P<0.01$, [f]$P<0.001$ attenuation caused by MK-801 compared to respective conditions in its absence.

labelling, whereas addition of memantine (100 μM) for 30, 60 or 90 min increased labelling by 83 ± 7%, 76 ± 5% and 74 ± 9% respectively, relative to basal incorporation rates (over 3 experiments).

Memantine increases [^{3}H]-inositol incorporation into all phosphoinositides

Memantine (100 μM) significantly increased [^{3}H]-inositol-labelling of PtdIns, PtdInsP and PtdInsP$_2$ (Table 2). It should be noted that this appears to be due to memantine affecting the rate of inositol phospholipid labelling rather than any effect on the absolute levels. Thus, exposure of slices to 100 μM memantine for 60 min, which in parallel experiments caused a 60% increase in [^{3}H]-PtdInsP$_2$ labelling, had no effect on PtdIns(4,5)P$_2$ mass (−memantine, 3138 ± 154; +memantine, 3082 ± 197 pmol per 25 μl slices: values for 3 experiments performed in duplicate).

In the absence of memantine, challenge with NMDA (1 mM) for 15 min resulted in similar levels of [^{3}H]-PtdIns labelling, but significantly decreased levels of both PtdInsP ($P<0.001$) and PtdInsP$_2$ ($P<0.01$) labelling, whilst in the presence of memantine (100 μM) enhanced levels of labelling of all inositol phospholipid species were well-maintained (Table 2). In both the absence and presence of memantine, carbachol stimulated [^{3}H]-inositol incorporation into all inositol phospholipids, reducing the differential between inositol phospholipid labelling observed ± memantine (Table 2). The increased levels of [^{3}H]-inositol phospholipid labelling observed in the presence of memantine led to a concomitant and proportional increase in NMDA-, but not carbachol-stimulated [^{3}H]-InsP$_x$ accumulation. Although it is possible that the increase in inositol phospholipid labelling seen in the presence of carbachol alone may have obscured any enhancement of [^{3}H]-InsP$_x$ accumulation in the presence of memantine, construction of carbachol concentration-response curves provided no evidence for this explanation (data not shown).

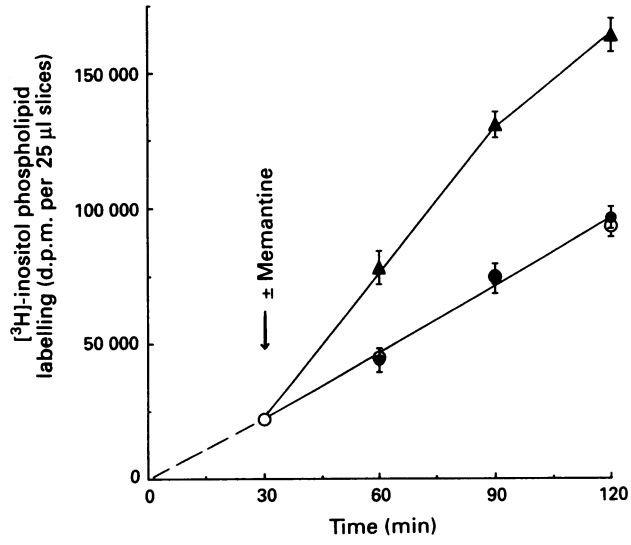

Figure 7 Effect of memantine and MK-801 on the time-courses of [^{3}H]-inositol incorporation into inositol phospholipids. Neonatal rat cerebral cortex slices were added to medium containing [^{3}H]-inositol for the indicated times. After 30 min, memantine (100 μM;▲), MK-801 (1 μM; ●) or vehicle (○) was added and incubations continued for the indicated times. Values are presented as means ± s.e.mean for a single representative experiment performed in triplicate. Similar effects of memantine and MK-801 were observed in two other experiments.

Taken together, these data suggest that NMDA and carbachol may stimulate phospholipase C activities which have differential access to the basal and memantine-enhanced [^{3}H]-inositol phospholipid pools.

Effect of memantine on basal and NMDA-stimulated isomer profiles of inositol (poly)phosphate accumulation

[^{3}H]-Ins(1/3)P$_1$ (by 187%), [^{3}H]-Ins(4)P$_1$ (by 983%) and [^{3}H]-Ins(1,4)P$_2$ (by 456%) accumulations were all increased by NMDA (1 mM), whilst the [^{3}H]-Ins(1,4,5)P$_3$ level decreased to below basal values. The latter finding is in good agreement with the effect of NMDA on basal Ins(1,4,5)P$_3$ mass levels shown in Figure 3a. Memantine (100 μM) did not significantly affect this isomer profile. NMDA evoked greater increases in [^{3}H]-Ins(1/3)P$_1$, [^{3}H]-Ins(4)P$_1$ and [^{3}H]-Ins(1,4)P$_2$ in the presence, compared to the absence of memantine (Table 3). Moreover, a significant NMDA-evoked increase in [^{3}H]-Ins(1,4,5)P$_3$ accumulation was observed and other [^{3}H]-inositol (poly)phosphate isomers were markedly increased in the presence of memantine, particularly those generated by activation of the 3-kinase route of Ins(1,4,5)P$_3$ metabolism. Thus, the increases in Ins(1,3,4,5)P$_4$ (14 fold), Ins(1,3,4)P$_3$ (3 fold), Ins(3,4)P$_2$ (3.5 fold) and Ins(1,3)P$_2$ (14 fold) were all greater than the approximate 2 fold increase which might be anticipated on the basis of differences in the [^{3}H]-inositol phospholipid fractions.

Discussion

Recent studies have provided considerable evidence to support the hypothesis that the therapeutic effects of memantine are mediated, at least in part, through the ability of this agent to inhibit transmembrane cation fluxes activated upon glutamate binding to NMDA-receptors (Kornhuber *et al.*, 1989; Bormann, 1989; Chen *et al.*, 1992; Parsons *et al.*, 1993). Here we have examined the ability of memantine to block the modulations of muscarinic cholinoceptor- and metabotropic glutamate receptor-stimulated phosphoinositide turnover by NMDA in neonatal rat cerebral cortex slices.

Table 2 Effects of memantine (100 μM) on basal and agonist-stimulated changes in phosphoinositide labelling and [^{3}H]-InsP$_x$ accumulation

Addition	*± memantine* (100 μM)	*[^{3}H]-PtdIns*	*[^{3}H]-PtdInsP*	*[^{3}H]-PtdInsP$_2$*	*[^{3}H]-InsP$_x$*
			(d.p.m. per 25 μl slices)		
–	–	56542 ± 1642	6881 ± 107	7929 ± 339	3674 ± 450
–	+	93036 ± 5157**	12495 ± 779***	11273 ± 925*	4440 ± 545
+ NMDA (1 mM)	–	63449 ± 2038	5051 ± 106	4740 ± 301	13359 ± 1688
	+	113085 ± 8502**	14894 ± 823***	11883 ± 1686**	26593 ± 2558**
+ CCh (1 mM)	–	88036 ± 3740	11719 ± 459	14486 ± 691	103237 ± 8437
	+	105454 ± 8023	15311 ± 852*	16498 ± 1515	99334 ± 7242

Data are presented as means ± s.e.mean for 3 separate experiments performed in triplicate. Indications of statistical significance are only shown for ± memantine comparisons with differences indicated as $*P<0.05$, $**P<0.01$ and $***P<0.001$. CCh = carbachol.

Table 3 Effect of memantine (100 μM) on basal and N-methyl-D-aspartate (NMDA)-stimulated accumulations of individual inositol (poly)phosphate isomers separated by h.p.l.c.

Isomer	*No addition*		*+NMDA* (1 mM)	
	– memantine	*+ memantine*	*– memantine*	*+ memantine*
Ins(1/3)P$_1$	7131 ± 599	8699 ± 1842	20464 ± 1356^c	50327 ± 4234^d
Ins(4)P$_1$	1722 ± 106	1792 ± 273	18657 ± 1372^c	39354 ± 2875^d
Ins(1,3)P$_2$	71 ± 13	73 ± 12	71 ± 4	1038 ± 143^d
Ins(1,4)P$_2$	1087 ± 261	1074 ± 115	6041 ± 567^b	13534 ± 575^e
Ins(3,4)P$_2$	46 ± 13	48 ± 6	61 ± 12	276 ± 40^d
Ins(1,3,4)P$_3$	521 ± 78	634 ± 27	425 ± 25	1683 ± 127^e
Ins(1,4,5)P$_3$	1604 ± 240	1982 ± 558	678 ± 59^a	4142 ± 382^e
Ins(1,3,4,5)P$_4$	393 ± 51	525 ± 48	386 ± 39	5752 ± 942^d

Neonatal rat cerebral cortex slices were labelled with [^{3}H]-inositol (2 μCi per vial) for 60 min. Data are presented as means ± s.e.mean for 3 separate experiments performed in triplicate. Indications of statistical significance are shown for +NMDA comparisons with differences indicated as $^aP<0.05$ for a decrease in the presence of NMDA and $^bP<0.01$, $^cP<0.001$ for increases in the presence of NMDA. Similar indications of statistical significance are shown for ± memantine comparisons with differences indicated as $^dP<0.01$, $^eP<0.001$ for increases in the presence of memantine compared to respective values in its absence.

In agreement with our previous work (Challiss *et al.*, 1994b), co-addition of NMDA markedly potentiated metabotropic glutamate receptor-stimulated [^{3}H]-InsP$_x$ accumulation. Thus, 10 μM NMDA increased the [^{3}H]-InsP$_x$ accumulation stimulated by 10 μM 1S,3R-ACPD at least 4 fold more than would be predicted if the responses to NMDA and 1S,3R-ACPD were simply additive (see inset to Figure 5). The potentiation of the metabotropic agonist-stimulated phosphoinositide response by 10 μM NMDA was concentration-dependently inhibited by both MK-801 (IC_{50} 68 nM) and memantine (IC_{50} 24 μM). Thus, like MK-801, memantine can antagonize the NMDA modulation of this response.

In another recent study, we demonstrated that high concentrations of NMDA profoundly inhibit carbachol-stimulated [^{3}H]-InsP$_x$ and Ins(1,4,5)P$_3$ mass accumulations (Challiss *et al.*, 1994a) and proposed that acute treatment of neonatal cerebral cortex slices with high concentrations of NMDA could cause progressive and irreversible damage, at least to cell populations responding to muscarinic cholinoceptor stimulation. Pretreatment of slices with memantine concentration-dependently reversed the inhibitory effect of 100 μM NMDA on carbachol-stimulated [^{3}H]-InsP$_x$ and Ins(1,4,5)P$_3$ mass accumulations, with 50% reversals of the NMDA effects being observed at 21 and 16 μM memantine respectively. If the inhibitory effects of NMDA are indeed causally linked to the excitotoxic actions of this agent, then the fact that memantine can block such effects is entirely consistent with the neuroprotective action of memantine reported by others (Seif el Nasr *et al.*, 1990; Erdö & Schaefer, 1991; Chen *et al.*, 1992; Keilhoff & Wolf, 1992; Weller *et al.*, 1993).

Taken together, these data provide further evidence that memantine can antagonize the actions of NMDA in a complex *in vitro* neuronal preparation. Although the concentrations of memantine required to antagonize the NMDA modulatory effects reported here are higher than values obtained in electrophysiological investigations (Bormann, 1989; Chen *et al.*, 1992; Parsons *et al.*, 1993) and competition binding assays (Kornhuber *et al.*, 1989), they are consistent with values obtained in more complex neuronal preparations such as the cortical wedge (Parsons *et al.*, 1993).

A number of previous studies have demonstrated that memantine may exert effects which might be unconnected with the established NMDA-receptor antagonist action of this agent (Maj, 1982), perhaps the most interesting of these being the ability of memantine to increase [^{3}H]-InsP$_x$ accumulation in a number of neuronal cell and slice preparations (Osborne & Quack, 1992). Such a dual activity (i.e. reducing glutamatergic neurotransmission via NMDA receptor blockade whilst exerting a 'metabotropic' action at the level of phosphoinositide cycle second messenger generation), may endow memantine with a number of additional, perhaps clinically beneficial, effects. For example, co-activation of metabotropic glutamate receptors can ameliorate the neurotoxic effects of inappropriate NMDA receptor activation (Koh *et al.*, 1991; Siliprandi *et al.*, 1992), possibly by inhibition of NMDA-gated cation fluxes through increasing diacylglycerol generation and activation of a protein kinase C-dependent phosphorylation of the NMDA receptor (Courtney & Nicholls, 1992).

In both neonatal and adult (Baird & Nahorski, 1991) rat cerebral cortex slices, NMDA alone can increase [^{3}H]-InsP$_x$ accumulation. That MK-801 can block the NMDA-evoked increase in [^{3}H]-InsP$_x$ accumulation strongly suggests that this effect is mediated through Ca^{2+} entry, via the integral cation channel of the NMDA receptor, leading to activation of Ca^{2+}-dependent phospholipase C activity. Although memantine also blocked [^{3}H]-InsP$_x$ accumulation stimulated by low

concentrations of NMDA it paradoxically potentiated [^{3}H]-$InsP_x$ accumulation stimulated by high concentrations of NMDA. Further experiments demonstrated memantine to have a second effect in the slice preparation, increasing the rate of [^{3}H]-inositol incorporation into the cellular phospholipid fraction. Although NMDA-evoked increases in [^{3}H]-$InsP_x$ accumulation in both the absence and presence of memantine (100 μM) were substantially antagonized by MK-801, this agent had no effect on the ability of memantine to increase [^{3}H]-inositol phospholipid labelling. Such data strongly suggest that the ability of memantine to enhance the rate of [^{3}H]-inositol incorporation into the phospholipid fraction is unrelated to NMDA receptor antagonism. Resolution of the [^{3}H]-inositol phospholipid fraction revealed that memantine increased labelling in all inositol phospholipid species approximately equally. However, these labelling effects were not paralleled by increases in PtdIns(4,5)P_2 mass which remained unaffected by incubation with 100 μM memantine for 60 min.

It might be inferred from these data that differences in agonist-stimulated [^{3}H]-$InsP_x$ accumulation in the absence and presence of memantine are generated by differences in the specific radioactivities of the inositol phospholipids and if it were possible to radiolabel slices with [^{3}H]-inositol to isotopic equilibrium such differences would no longer be observed. However, this is unlikely to explain the results of Osborne & Quack (1992), who assessed memantine-stimulated [^{3}H]-$InsP_x$ accumulations, not only in acutely labelled slice preparations, but also in retinal cell cultures labelled with [^{3}H]-inositol for 16 h to achieve equilibrium labelling. Furthermore, other findings have arisen from the current investigation to suggest that memantine may produce effects over and above those resulting from a simple facilitation of the rate of inositol phospholipid labelling in neonatal cerebral cortex slices. Thus, although the increase in the NMDA-evoked [^{3}H]-$InsP_x$ accumulation was in proportion with the increase in [^{3}H]-inositol phospholipid labelling in the presence of memantine, the same was not true of the carbachol-stimulated response. This discrepancy was also evident when the magnitude of the [^{3}H]-$InsP_x$ accumulations was equalized by adjustment of agonist concentration (1 mM NMDA versus 3 μM carbachol). One interpretation of these data is that memantine selectively facilitates [^{3}H]-inositol incorporation into an inositol phospholipid pool which is accessible to the phospholipase C(s) activated by NMDA, but not by the enzyme activated by carbachol. Such a possibility is not without precedent. Mn^{2+} has been shown to enhance greatly [^{3}H]-inositol incorporation into inositol phospholipids in different neuronal preparations (Yandrasitz & Segal, 1979; Schoepp, 1985). However, although the increased phospholipid radiolabelling is reflected in increased basal [^{3}H]-$InsP_x$ accumulations in cerebral cortex slices, agonist-stimulated responses are largely unaffected suggesting that Mn^{2+} facilitates [^{3}H]-inositol incorporation into a phospholipid pool inaccessible to agonist-stimulated phospholipase C activity (Schoepp, 1985).

Another important observation regarding the possible actions of memantine has been made from the detailed analysis of individual inositol (poly)phosphate isomers generated under basal and agonist-stimulated conditions. In agreement with data presented by Baird & Nahorski (1991), NMDA significantly increased the accumulations of Ins(1,4)P_2, Ins(4)P_1 and Ins(1/3)P_1, whilst having no effect or decreasing the accumulations of higher inositol phosphates. These data suggest that NMDA may cause the selective activation of one or more isozymes of phospholipase C which preferentially hydrolyses PtdIns(4)P and PtdIns. Memantine profoundly affected the profile of NMDA-stimulated inositol (poly)phosphate accumulation. In the presence of memantine, NMDA evoked increases in Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 accumulations as well as substantial increases in Ins(1,3,4)P_3, Ins(1,3)P_2 and Ins(3,4)P_2 which were all unchanged from basal values during NMDA challenge in the absence of memantine.

These data suggest that memantine can influence the routes of inositol polyphosphate synthesis and degradation, promoting substantial increases in the concentrations of the established and putative second messengers Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4. Such a modulatory effect can only be brought about if memantine directly or indirectly causes a substantial shift in the substrate selectivity of NMDA-stimulated phospholipase C activities towards hydrolysis of PtdIns(4,5)P_2. It is possible that activation of the 3-kinase route of Ins(1,4,5)P_3 metabolism might also be affected by memantine; however, the increase in Ins(1,4,5)P_3 concentration in the presence of an NMDA-evoked elevation of $[Ca^{2+}]_i$ may be sufficient to increase the flux through this enzyme (Shears, 1991).

In summary, the present study has demonstrated that memantine concentration-dependently antagonized both stimulatory and inhibitory modulations of agonist-stimulated phosphoinositide turnover by NMDA, consistent with previous reports that memantine is an uncompetitive blocker of the integral cation channel of the NMDA receptor complex. In addition, we have provided evidence for two further actions of memantine. Memantine stimulates the rate at which [^{3}H]-inositol is incorporated into PtdIns, PtdIns(4)P and PtdIns(4,5)P_2. The fact that the enhanced labelling of [^{3}H]-PtdInsP_2 was not reflected in a significant increase in PtdIns(4,5)P_2 mass, suggests that memantine accelerates the rate of inositol incorporation into the phosphoinositide pool rather than perturbing the steady-state levels of these membrane lipids. Whether this reflects the ability of memantine to stimulate selectively or generally inositol:inositol phospholipid exchange, and thus facilitate the recovery of membrane phosphoinositide pools following depletion (Monaco & Adelson, 1991; Thomas *et al.*, 1993) remains to be addressed. Finally, although memantine alone does not increase phosphoinositide turnover, it does dramatically alter the profile of NMDA-stimulated inositol (poly)phosphate generation. Our data strongly argue that memantine not only facilitates NMDA-stimulated [^{3}H]-$InsP_x$ accumulation but also shifts the substrate hydrolysed by the activated phospholipase C(s) from PtdIns and PtdIns(4)P substantially towards PtdIns-(4,5)P_2. Therefore, in addition to the therapeutic advantages memantine has been argued to possess on the basis of studies of the kinetics of NMDA-receptor/channel blockade (Chen *et al.*, 1992; Parsons *et al.*, 1993), further independent loci of action, which may be of significance in the known therapeutic actions and envisaged therapeutic potentials of memantine, should also be taken into account.

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References

BAIRD, J.G. & NAHORSKI, S.R. (1991). Stimulatory and inhibitory effects of N-methyl-D-aspartate on ^{3}H-inositol polyphosphate accumulation in rat cortical slices. *J. Neurochem.*, **57**, 629–635.

BATTY, I.H., LETCHER, A.J. & NAHORSKI, S.R. (1989). Accumulation of inositol polyphosphate isomers in agonist-stimulated cerebral cortex slices. *Biochem. J.*, **258**, 23–32.

BORMANN, J. (1989). Memantine is a potent blocker of N-methyl-D-aspartate (NMDA) receptor channels. *Eur. J. Pharmacol.*, **166**, 591–592.

CHALLISS, R.A.J., BATTY, I.H. & NAHORSKI, S.R. (1988). Mass measurements of inositol 1,4,5-trisphosphate in rat cerebral cortical slices using a radioreceptor assay: effects of neurotransmitters and depolarization. *Biochem. Biophys. Res. Commun.*, **157**, 684–691.

CHALLISS, R.A.J., MISTRY, R., GRAY, D.W. & NAHORSKI, S.R. (1994a). Modulation of muscarinic cholinoceptor-stimulated inositol 1,4,5-trisphosphate accumulation by N-methyl-D-aspartate in neonatal rat cerebral cortex. *Neuropharmacology*, **33**, 15–25.

CHALLISS, R.A.J., MISTRY, R., GRAY, D.W. & NAHORSKI, S.R. (1994b). Modulatory effects of NMDA on phosphoinositide responses evoked by the metabotropic glutamate receptor agonist 1S,3R-ACPD in neonatal rat cerebral cortex. *Br. J. Pharmacol.*, **112**, 231–239.

CHALLISS, R.A.J. & NAHORSKI, S.R. (1993). Measurement of inositol 1,4,5-trisphosphate, inositol 1,3,4,5-tetrakisphosphate, and phosphatidylinositol 4,5-bisphosphate in brain. *Methods Neurosci.*, **18**, 224–244.

CHEN, H.-S.V., PELLEGRINI, J.W., AGGARWAL, S.K., LEI, S.Z., WARACH, S., JENSEN, F.E. & LIPTON, S.A. (1992). Open-channel block of NMDA responses by memantine: therapeutic advantage against NMDA receptor-mediated neurotoxicity. *J. Neurosci.*, **12**, 4427–4436.

COURTNEY, M.J. & NICHOLLS, D.G. (1992). Interactions between phospholipase C-coupled and N-methyl-D-aspartate receptors in cultured cerebellar granule cells: protein kinase C mediated inhibition of N-methyl-D-aspartate responses. *J. Neurochem.*, **59**, 983–992.

DITZLER, K. (1991). Efficacy and tolerability of memantine in patients with dementia syndrome. *Arzneim. Forsch./Drug Res.*, **41**, 773–780.

DOWNES, C.P. & WUSTEMAN, M.M. (1983). Breakdown of polyphosphoinositides and not phosphatidylinositol accounts for the muscarinic agonist stimulated inositol phospholipid metabolism in rat parotid gland. *Biochem. J.*, **216**, 633–640.

ERDÖ, S.L. & SCHAEFER, M. (1991). Memantine is highly potent in protecting cortical cultures against excitotoxic cell death evoked by glutamate and NMDA. *Eur. J. Pharmacol.*, **198**, 215–217.

GOERTELMEYER, R. & ERBLER, H. (1992). Memantine in the treatment of mild to moderate dementia syndrome. A double-blind placebo-controlled study. *Arzneim. Forsch./Drug Res.*, **42**, 904–913.

GROSSMANN, W. & SCHUTZ, W. (1982). Memantine and neurogenic bladder dysfunction in spastic conditions. *Arzneim. Forsch./Drug Res.*, **32**, 1273–1276.

KEILHOFF, G. & WOLF, G. (1992). Memantine prevents quinolinic acid-induced hippocampal damage. *Eur. J. Pharmacol.*, **219**, 451–454.

KOH, J.-Y., PALMER, E. & COTMAN, C.W. (1991). Activation of the metabotropic glutamate receptor attenuates N-methyl-D-aspartate neurotoxicity in cortical cultures. *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 9431–9435.

KORNHUBER, J., BORMANN, J., RETZ, W., HUEBERS, M. & RIEDERER, P. (1989). Memantine displaces [^{3}H]MK-801 at therapeutic concentrations in postmortem human frontal cortex. *Eur. J. Pharmacol.*, **166**, 589–590.

LUPP, A., LUECKING, C.H., KOCH, R., JACKISCH, R. & FEUERSTEIN, T.J. (1992). Inhibitory effects of the antiparkinsonian drugs memantine and amantidine on NMDA-evoked acetylcholine release in rabbit caudate nucleus *in vitro*. *J. Pharmacol. Exp. Ther.*, **263**, 717–724.

MAJ, J. (1982). Die Wirkung von Memantin auf zentrale Neurotransmittersysteme. Eine Zusammenfassung der Ergebnisse. *Arzneim. Forsch./Drug Res.*, **32**, 1256–1259.

MAJ, J., SOWINSKA, H., BARAN, L. & SARNEK, J. (1974). Pharmacologic effects of 1,3-dimethyl-5-amino-adamantane, a new adamantane derivative. *Eur. J. Pharmacol.*, **26**, 9–14.

MONACO, M.E. & ADELSON, J.R. (1991). Evidence for coupling of resynthesis to hydrolysis in the phosphoinositide cycle. *Biochem. J.*, **279**, 337–341.

OSBORNE, N.N. & QUACK, G. (1992). Memantine stimulates inositol phosphates production in neurones and nullifies NMDA-induced destruction of retinal neurones. *Neurochem. Int.*, **21**, 329–336.

PARSONS, C.G., GRUNER, R., ROZENTAL, J., MILLAR, J. & LODGE, D. (1993). Patch clamp studies on the kinetics and selectivity of NMDA-receptor antagonism by memantine (1-amino-3,5-dimethyladmantan). *Neuropharmacology*, **32**, 1337–1350.

SCHNEIDER, E., FISCHER, P.A., CLEMENS, R., BALZEREIT, F., FUENFGELD, E.W. & HAASE, H.J. (1984). Effects of oral memantine on symptoms of Parkinson's disease. *Deutsche Med. Wochenzeitschr.*, **109**, 987–990.

SCHOEPP, D. (1985). Manganese stimulates the incorporation of [^{3}H]inositol into a pool of phosphatidylinositol in brain that is not coupled to agonist-induced hydrolysis. *J. Neurochem.*, **45**, 1481–1486.

SEIF EL NASR, M., PERUCHE, B., ROSSBERG, C., MENNEL, H.D. & KRIEGLSTEIN, J. (1990). Neuroprotective effect of memantine demonstrated *in vivo* and *in vitro*. *Eur. J. Pharmacol.*, **185**, 19–24.

SHEARS, S.B. (1991). Regulation of the metabolism of 1,2-diacylglycerols and inositol phosphates that respond to receptor activation. *Pharmacol. Ther.*, **49**, 79–104.

SILIPRANDI, R., LIPARTITI, M., FADDA, E., SAUTTER, J. & MANEV, H. (1992). Activation of the glutamate metabotropic receptor protects retina against N-methyl-D-aspartate toxicity. *Eur. J. Pharmacol.*, **219**, 173–174.

SIMPSON, C.M.F., BATTY, I.H. & HAWTHORNE, J.N. (1987). Physiological responses to receptor activation: phosphoinositide turnover. In *Neurochemistry – a Practical Approach* ed. Turner, A.J. & Bachelard, H.S. pp. 193–224, Oxford: IRL Press.

THOMAS, G.M.T., CUNNINGHAM, E., FENSOME, A., BALL, A., TOTTY, N.F., TRUONG, O., HSUAN, J.J. & COCKCROFT, S. (1993). An essential role for phosphatidylinositol transfer protein in phospholipase C-mediated inositol lipid signalling. *Cell*, **74**, 919–928.

WELLER, M., FINIELSMARLIER, F. & PAUL, S.M. (1993). NMDA receptor-mediated glutamate toxicity of cultured cerebellar, cortical and mesencephalic neurons: neuroprotective properties of amantidine and memantine. *Brain Res.*, **613**, 143–148.

WESEMANN, W., SONTAG, K.H. & MAJ, J. (1983). On the pharmacodynamics and pharmacokinetics of memantine. *Arzneim. Forsch./Drug Res.*, **33**, 1122–1134.

YANDRASITZ, J.R. & SEGAL, S. (1979). The effect of $MnCl_2$ on the basal and acetylcholine-stimulated turnover of phosphatidylinositol in synaptosomes. *FEBS Lett.*, **108**, 279–282.

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