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(*S*)-2,3-dihydro-[3,4]cyclopentano-1,2,4-benzothiadiazine-1,1-dioxide: (S18986-1) a positive modulator of AMPA receptors enhances (*S*)-AMPA-mediated [^{3}H]noradrenaline release from rat hippocampal and frontal cortex slices

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This paper is dedicated to the memory of Matthieu Closier.

Abstract

The present study describes the effect of (*S*)-2,3-dihydro-[3,4]cyclopentano-1,2,4-benzothiadiazine-1,1-dioxide (S18986-1), a positive allosteric modulator of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors with cognitive-enhancing effects, on (*S*)-AMPA-induced [^{3}H]noradrenaline release in rat hippocampal and frontal cortex slices. (*S*)-AMPA significantly increased [^{3}H]noradrenaline release in rat hippocampus and frontal cortex slices, whereas S18986-1 (3–1000 μM) alone, was inactive. However, S18986-1 between 30 and 1000 μM potently enhanced (+200%) (*S*)-AMPA-mediated [^{3}H]noradrenaline release in both hippocampal and frontal cortex slices. The capacity of S18986-1 to potentiate [^{3}H]noradrenaline release was specific for AMPA receptors as S18986-1 failed to potentiate either kainate and *N*-methyl-D-aspartate (NMDA)-mediated release of [^{3}H]noradrenaline in rat hippocampal slices. Moreover, 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[*f*]quinoxaline-7-sulfonamide (NBQX) and 1-(4-aminophenyl)-3-methylcarbamoyl-4-methyl-3,4-dihydro-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (GYKI-53655) but not (5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine ((+)-MK-801), inhibited (*S*)-AMPA and S18986-induced stimulation of (*S*)-AMPA-mediated [^{3}H]noradrenaline release. In addition, S18986-1-induced stimulation of (*S*)-AMPA-evoked [^{3}H]noradrenaline release was markedly attenuated in the presence of tetrodotoxin (1 μM) and in Ca^{2+}-free buffer. S18986-1 enhanced (*S*)-AMPA-mediated [^{3}H]noradrenaline release to a greater extent than its corresponding (*R*)-enantiomer S19024-1 and racemic mixture S17951-1. However, positive allosteric modulators of AMPA receptors such as aniracetam failed to potentiate AMPA-mediated noradrenaline release in hippocampal slices, whereas cyclothiazide potently enhanced (*S*)-AMPA-mediated [^{3}H]noradrenaline release. These results suggest that the capacity of S18986-1 to enhance AMPA receptor-mediated release of noradrenaline in rat hippocampus and frontal cortex, could contribute to the cognition enhancing mechanisms of S18986-1.

Keywords: AMPA receptor; S18986-1; Hippocampus; Noradrenaline release; Allosteric modulator

1. Introduction

Extensive pharmacology of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor has defined competitive quinoxaline-type antagonists such as 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[*f*]quinoxaline-7-sulfonamide (NBQX) (Honore et al., 1988) acting at the glutamate recognition site, or non-competitive antagonists, such as 1-(4-aminophenyl)-3-methylcarbamoyl-4-methyl-3,4-dihydro-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (GYKI-53655), acting at the 2,3-benzodiazepine site (Vizi et al., 1996). Additional compounds capable of prolonging and enhancing AMPA-mediated currents by inhibiting receptor desensitization including the nootropic agents (aniracetam), the ampakine 1-(quinolalin-6-ycarbonyl)-piperidine (CX-516) or the benzothiadiazides; cyclothiazide, diazoxide and 7-chloro-3-methyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine *S,S*-dioxide (IDRA-21) have been identified (Ito et al., 1990; Randle et al., 1993; Yamada and Tang, 1993). Moreover, electrophysiological (Seki-

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guchi et al., 1998) and autoradiographic analysis (Kessler et al., 1998) suggests distinct regional and molecular effects for these ligands as regards the enhancement of AMPA receptor activation. For example, cyclothiazide appears to have greater selectivity towards the 'flip' isoform, whereas CX-516 and aniracetam appear to have a modest preference for 'flop' variants (Partin et al., 1994).

Post-synaptic AMPA receptors appear to play a role in regulating long-term potentiation, a physiological process which may represent a molecular mechanism for learning and memory (Bliss and Collingridge, 1993). Moreover, the potential role of AMPA receptors in both learning and memory processes (Staubli et al., 1994) suggests that mechanisms leading to the enhancement of AMPA receptor-mediated effects could have therapeutic potential in attentional and/or cognitive disorders linked to a hypofunction of glutamatergic systems (Parsons et al., 1998). Indeed, previous studies have shown that IDRA-21 improved the performance of rats in the Morris water maze, and passive avoidance tests after scopolamine or NBQX-mediated amnesia (Zivkovic et al., 1995; Thompson et al., 1995), antagonised alprazolam-mediated cognitive deficits in monkeys (Thompson et al., 1995) and facilitated the induction of long-term potentiation in rat hippocampal slices (Arai et al., 1996). Similarly, CX-516 was capable of improving performance in several rodent models of learning-dependent tasks (Granger et al., 1993; Staubli et al., 1994; Larson et al., 1995; Granger et al., 1996; Davis et al., 1997; Hampson et al., 1998), and recent evidence suggested that CX-516 improved ''delayed recall'' in aged (64–76 years old) human volunteers (Lynch et al., 1997).

The potential role of presynaptic AMPA receptors in the release of several neurotransmitters implicated in cognitive processes including, acetylcholine (Moor et al., 1996; Jin, 1997; Giovannini et al., 1998; Bonhomme et al., 1999), glutamate (Barnes et al., 1994; Dakshaben and Croucher, 1997), γ-aminobutyric acid (GABA) (Giovannini et al., 1998) and noradrenaline (Jin, 1997) in different rat brain structures have been established. It is therefore likely that the cognition-enhancing properties of compounds capable of positively modulating AMPA receptors could, in addition to their effects on long-term potentiation (Arai et al., 1996), also reside in their ability to prolong AMPA-receptor-mediated control of neurotransmitter release. Indeed, positive modulators of AMPA receptors such as 1-(1,3-benzodioxol-5-ylcarbonyl)piperidine (1-BCP) and cyclothiazide, potentiated AMPA-mediated release of [^{3}H]noradrenaline in rat hippocampal slices (Desai et al., 1994, 1995; Cowen and Beart, 1998; Pittaluga et al., 1997, 1999), and AMPA-mediated release of [^{3}H]-dopamine in rat striatal slices (Jin, 1997).

(*S*)-2,3-dihydro-[3,4]cyclopentano-1,2,4-benzothiadiazine-1,1-dioxide (S18986-1), a selective AMPA receptor modulator, selectively potentiated AMPA-induced currents in Xenopus oocytes injected with polyA(+)mRNA of rat cerebral cortex (Desos et al., 1996) and increased the amplitude of excitatory post-synaptic field potentials (e.p.s.f.p.s) in the CA1 region of rat hippocampal slices in the presence of Mg^{2+} (Lepagnol et al., 1997). Moreover, S18986-1 was capable of prolonging the duration of long-term potentiation in the dentate gyrus of anaesthetised rats (Lepagnol et al., 1997). In terms of cognition-enhancing effects, S18986-1 prevented scopolamine-induced amnesia in a passive avoidance test, and was active in the object recognition test in both young and aged rats (Lepagnol et al., 1997; Lebrun et al., 1998, 2000). Consequently, based on previous reports (Desai et al., 1994, 1995; Cowen and Beart, 1998; Pittaluga et al., 1997, 1999), and in order to further identify potential mechanism(s) by which S18986-1 may manifest cognition enhancing effects, the capacity of S18986-1 to enhance (*S*)-AMPA-evoked [^{3}H]noradrenaline release in rat hippocampal and frontal cortex slices was investigated. Preliminary results on S18986-1-mediated potentiation of (*S*)-AMPA-evoked [^{3}H]noradrenaline release in rat hippocampal slices was previously presented in abstract form (Lockhart et al., 1998).

2. Materials and methods

2.1. Materials

[^{3}H]noradrenaline (specific activity: 48 Ci/mmol) was obtained from Amersham Laboratories. Kainate, aniracetam, *N*-methyl-D-aspartate (NMDA), tetrodotoxin, NBQX, and (5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5H-dibenzo-[*a*,*d*]cyclohepten-5,10-imine ((+)-MK-801) hydrogen maleate, were obtained from RBI, Sigma France. Cyclothiazide, (*S*)-AMPA were supplied by Tocris-Bioblock, France. S18986-1, S19024-1 the corresponding ((*R*)-enantiomer, and S17951-1 the equivalent ((±)-racemic mixture, and GYKI-53655 were supplied by Dr. Alex Cordi (Institut de Recherches Servier).

2.2. Methods

2.2.1. Preparation of cerebral tissue slices and assay of [^{3}H]noradrenaline release

The measurement of [^{3}H]noradrenaline release from rat hippocampal and cortical slices, respectively, was carried out according to the method of Desai et al. (1995). Male Wistar rats (240–260 g) were decapitated and the brain removed and the hippocampi or frontal cortices dissected. The tissue was cross-chopped with a McIlwain tissue slicer (300 × 300 μm) and slices were rinsed in Krebs solution (118 mM NaCl, 4 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 24 mM $NaHCO_3$, 1.12 mM KH_2PO_4, 10 mM D-Glucose, 0.01 mM Pargyline, pH 7.4) equilibrated with 95% O_2–5% CO_2. Slices were then incubated with Krebs containing [^{3}H]noradrenaline (0.16 μCi/ml) for 30 min at 37°C. The tissue was then rinsed several times with fresh Krebs solution, and 300 μl aliquots of tissue slices were

placed between Whatman GF-B filter discs in the reaction chambers of a B18 Superfusion System (Brandel).

The tissue was superfused with Krebs buffer for 45–60 min with a constant flow rate of 0.3 ml/min. The superfusate was then collected in 5 min fractions (1.5 ml/fraction). The first four fractions were perfused with Krebs buffer alone, compounds under study (S18986-1 or analogues) were perfused at fraction #5 (receptor antagonists were added at the same time as S18986-1), (*S*)-AMPA was then added at fraction #6 followed by perfusion with Krebs buffer up to fraction #12. At the end of the study, the filter and tissue were removed and dissolved overnight in tissue solubiliser (Soluene-350, Packard). The quantity of radioactivity (dpm) was estimated for each fraction and for the tissue sample with a Beckman scintillation counter.

The fractional release was estimated as the percentage of radioactivity present in each fraction relative to the total radioactivity from all fractions plus the tissue sample. The data are expressed as the fractional release of [^{3}H]noradrenaline release from fractions #7, 8, 9 and 10 relative to the total in all fractions plus the tissue.

2.2.2. Statistical analysis

Data (percentage fractional release) were analysed by either one-way analysis of variance (ANOVA) with a complementary Dunnett's test or two-way ANOVA followed by different complementary analysis (Newman–Keuls, contrast method) as detailed in the respective figure legends. EC_{50} values were determined by non-linear regression analysis (GraphPad PRISM™).

3. Results

1.1. S18986-1-mediated enhancement of (S)-AMPA evoked [^{3}H]noradrenaline release in rat brain slices

In general, basal release of tritium from rat hippocampal slices, preloaded with [^{3}H]noradrenaline, corresponded to a percentage fractional release per fraction of approximately 0.4% of total tissue radioactivity during the entire duration of the perfusion (Fig. 1). In addition, S18986-1 (250 μM) or dimethyl sulphoxide (DMSO) (1% v/v), added at fraction #5, did not induce any significant release of [^{3}H]noradrenaline during the perfusion procedure (Fig. 1). Exposure of rat hippocampal slices to (*S*)-AMPA for 5 min at 100 μM resulted in a maximal fractional release of ~1.4% in fractions #7 and #8 (Fig. 1). Combined fractional release estimates of (*S*)-AMPA-mediated [^{3}H]noradrenaline release, calculated for fractions #7–10, showed that (*S*)-AMPA between 30 and 1000 μM significantly increased [^{3}H]noradrenaline release (+170% to +265%), compared to basal release (EC_{50} = 20.4 μM (12.2–28.6)), with maximal effects observed at concentrations ranging from 100 to 1000 μM (Fig. 2a). S18986-1, failed to provoke any significant [^{3}H]noradrenaline release in rat hippocampal slices between 0.03 and 0.3 mM, although a moderate but significant increase (+42%) was observed at 1000 μM compared to DMSO controls (Fig. 2b).

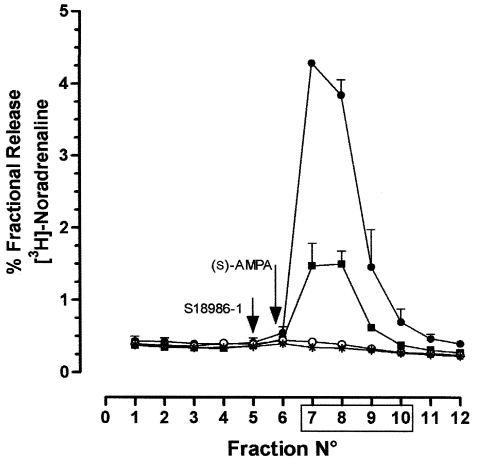

Fig. 1. Effect of (*S*)-AMPA and S18986-1 on [^{3}H]noradrenaline release from hippocampal slices. Slices were prelabelled with [^{3}H]noradrenaline and continuously perfused with buffer for 45–60 min with a constant flow rate of 0.3 ml/min, and the superfusate was collected in 5 min fractions (1.5 ml/fraction). The first four fractions were perfused with buffer alone, S18986-1 was perfused at fraction #5, (*S*)-AMPA at fraction #6 followed by perfusion with buffer up to fraction #12. The quantity of radioactivity (dpm) was estimated for each fraction and for the tissue sample and expressed as percentage fractional release. Basal (-∗-); S18986-1 (250 μM) (-○-); (*S*)-AMPA (100 μM) (-■-); S18986-1+(*S*)-AMPA (-●-).

In order to assess the potential of S18986-1 to enhance (*S*)-AMPA-mediated release of [^{3}H]noradrenaline in rat hippocampus, slices were initially perfused for 5 min with S18986-1 (3–1000 μM) followed by (*S*)-AMPA at 100 μM, corresponding to the concentration of agonist producing its maximal effect on [^{3}H]noradrenaline release. S18986-1 considerably enhanced (*S*)-AMPA-evoked [^{3}H]noradrenaline release with maximal effects observed at fractions #7and #8 (Fig. 1). Moreover, S18986-1 (0.03–1 mM) significantly stimulated ($P < 0.001$), in a concentration-dependent manner (EC_{2X} ~125 μM), (*S*)-AMPA-mediated release of [^{3}H]noradrenaline (Fig. 2b). (*S*)-AMPA-mediated release of [^{3}H]noradrenaline was significantly increased with S18986-1 concentrations of 100 μM (+76%), 300 μM (+160%) or 1 mM (+242%) (Fig. 2b). In the same context, increasing concentrations of (*S*)-AMPA (10–1000 μM) resulted in a concentration-dependent increase (EC_{50} = 38.4 μM (25.4–51.3); $P < 0.001$) in the capacity of S18986-1 (250 μM) to potentiate (*S*)-AMPA-mediated [^{3}H]noradrenaline release in rat hippocampal slices (Fig. 2a). S18986-1 (250 μM) produced an approximate +80% to +143% increase in (*S*)-AMPA-evoked [^{3}H]noradrenaline release, at (*S*)-AMPA concentrations ranging from 10 to 100 μM, with maximal stimulation (+170% to +210%) observed between (*S*)-AMPA concentrations of 0.3–1 mM (Fig. 2a).

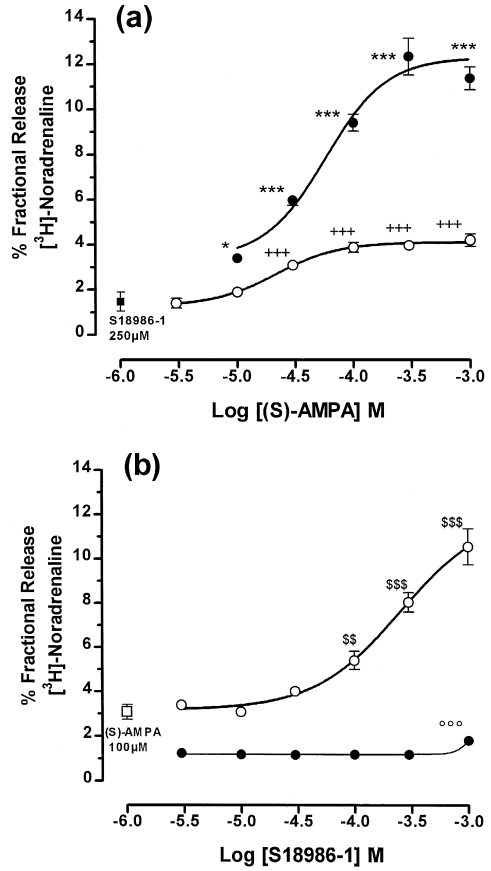

Fig. 2. Concentration-dependent effect of (*S*)-AMPA and S18986-1 on [^{3}H]noradrenaline release. (a) Prelabelled hippocampal slices were perfused at fraction #6 with different concentrations of (*S*)-AMPA (3–1000 μM) (-○-). In a parallel study, slices were perfused at fraction #5 with S18986-1 (250 μM) alone (-■-) or at fraction #6 in the presence of different concentrations of (*S*)-AMPA (10–1000 μM) (-●-). (b) Hippocampal slices were perfused at fraction #5 with different concentrations of S18986-1 (3–1000 μM) (-●-) or at fraction #6 with (*S*)-AMPA (100 μM) alone (-□-) or in the presence of different concentrations of S18986-1 (3–1000 μM) (-○-). Data: mean ± S.E.M.; $n = 6$. Analysis: one-way ANOVA with complementary Dunnett's test; ((*S*)-AMPA vs. DMSO; $^{+++}P < 0.001$); (S18986-1 vs. (*S*)-AMPA + S18986-1; $^{*}P < 0.05$, $^{***}P < 0.001$); (S18986-1 vs. DMSO; $^{\circ\circ\circ}P < 0.001$); ((*S*)-AMPA vs. (*S*)−(*S*)-AMPA + S18986-1; $^{\$\$}P < 0.01$, $^{\$\$\$}P < 0.001$).

In a comparative study, (*S*)-AMPA induced [^{3}H]noradrenaline release in rat frontal cortex slices, to a similar extent as hippocampal slices (Fig. 3). In frontal cortex, S18986-1 (250 μM) enhanced (*S*)-AMPA-mediated [^{3}H]-NE release at (*S*)-AMPA concentrations of 30 μM (+102%), 100 μM (+57%), and 300 μM (+71%) (Fig. 3a), whereas in hippocampal slices S18986-1 enhanced (*S*)-AMPA-mediated release of [^{3}H]noradrenaline, at (*S*)-AMPA concentrations of 30 μM (+97%), 100 μM (+85%), and 300 μM (+126%) (Fig. 3b).

3.2. Characterisation of S18986-1 enhancement of (S)-AMPA-evoked [^{3}H]noradrenaline release in hippocampus

In order to evaluate the specificity of S18986-1 at stimulating (*S*)-AMPA-mediated [^{3}H]noradrenaline release in hippocampal slices its capacity to stimulate neurotransmitter release induced via kainate and NMDA receptors was examined (Fig. 4). In this aim, under similar conditions as described for (*S*)-AMPA (see Fig. 3), S18986-1 failed to potentiate either kainate ($^{NS}P = 0.236$) or NMDA ($^{NS}P = 0.948$)-receptor-mediated [^{3}H]noradrenaline release, at equivalent concentrations of agonist (Fig. 4a and b). Moreover, the failure of (+)-MK-801 to inhibit both (*S*)-AMPA and S18986-1-mediated potentiation of (*S*)-AMPA induced [^{3}H]noradrenaline release in hippocampal slices further supported the lack of involvement of NMDA receptors (Fig. 5a). The competitive AMPA receptor antagonist, NBQX significantly inhibited (*S*)-AMPA-induced [^{3}H]noradrenaline release (−75%: $P < 0.001$) (Fig. 5b), whereas GYKI-53655 a non-competitive AMPA antagonist, acting at the 2,3-benzodiazepine site, only partially inhibited [^{3}H]noradrenaline release at the concentration tested, (−43%; P = NS) (Fig. 5c). However, both antagonists significantly reduced S18986-1-mediated potentiation

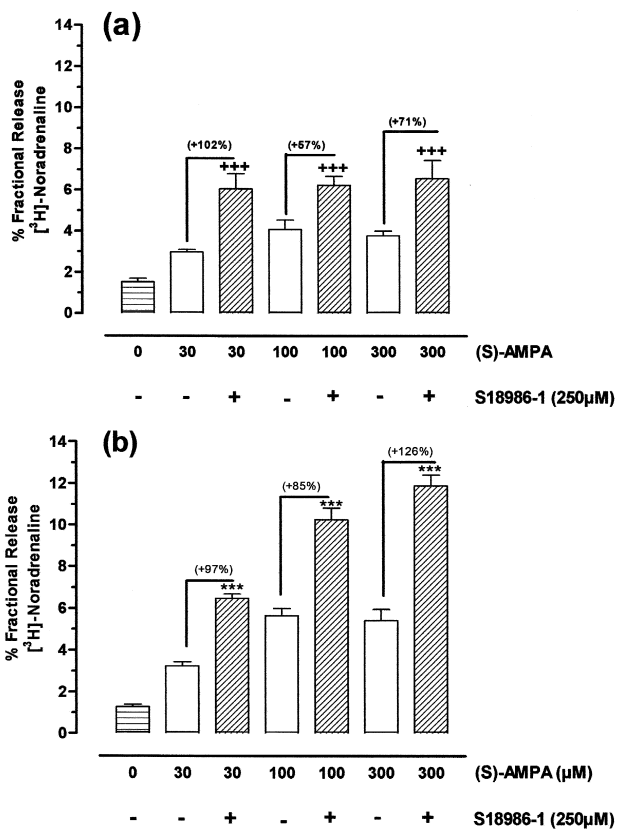

Fig. 3. Effect of S18986-1 on (*S*)-AMPA-stimulated [^{3}H]noradrenaline release in rat hippocampal and frontal cortex slices. (a) Rat frontal cortex or (b) hippocampal slices were perfused at fraction #5 with either buffer alone or S18986-1 (250 μM) followed at fraction #6 by either (*S*)-AMPA at 30, 100, and 300 μM. Data: mean ± S.E.M.; $n = 4$–6. Analysis: two-way ANOVA with a complementary comparison's test (Frontal cortex: S18986-1 vs. S18986-1 + (*S*)-AMPA, at all pooling levels; $^{+++}P < 0.001$); (Hippocampus: S18986-1 vs. S18986-1 + (*S*)-AMPA, at all pooline levels; $^{***}P < 0.001$).

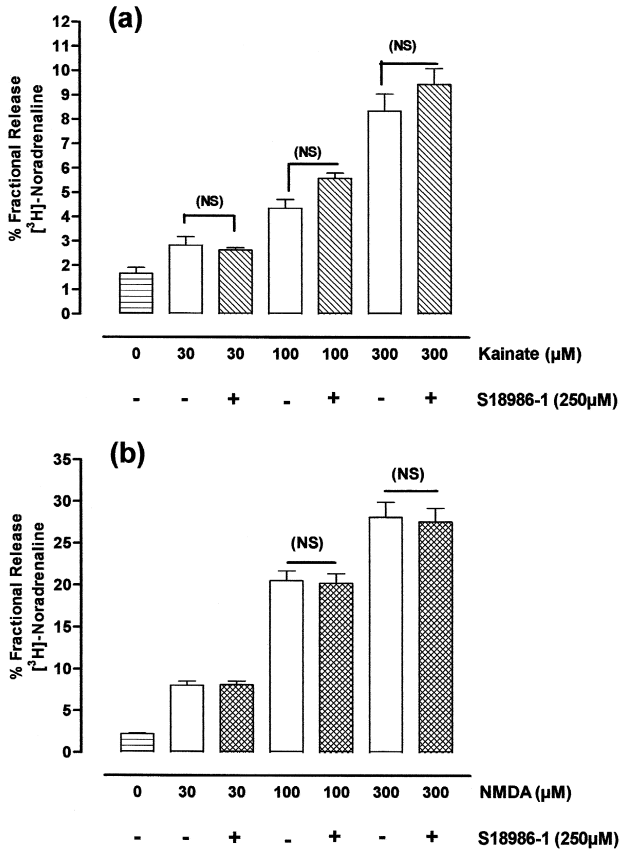

Fig. 4. Effect of S18986-1 on kainate or NMDA-stimulated [^{3}H]noradrenaline release. Hippocampal slices were perfused at fraction #5 with either buffer alone or S18986-1 (250 μM) followed at fraction #6 by either (a) kainate (b) NMDA (in Mg^{2+}-free buffer) at 30, 100, and 300 μM. Data: mean ± S.E.M.; $n = 4–8$. Analysis: two-way ANOVA (DMSO vs. kainate or NMDA, at all pooling levels; $^{***}P < 0.001$), (S18986-1 vs. S18986-1 + kainate or NMDA, at all pooling levels; $P > 0.05$).

of [^{3}H]noradrenaline release although NBQX was more potent (−89%) compared to GYKI-53655 (−61%) at equivalent concentrations (Fig. 5a and b).

Inhibition of action potential propagation with the Na^+-channel blocker tetrodotoxin (Tetrodotoxin) at 1 μM, partially reduced basal [^{3}H]noradrenaline release (−34%; P = NS) from hippocampal slices (Fig. 6a). Moreover, tetrodotoxin significantly inhibited both (*S*)-AMPA-induced [^{3}H]noradrenaline release (−86%; $P < 0.001$), and S18986-1-mediated enhancement (−57%; $P < 0.001$) of neurotransmitter release in hippocampal slices (Fig. 6a). In calcium-free Krebs buffer, (*S*)-AMPA (30–300 μM) did not significantly stimulate [^{3}H]noradrenaline release in rat hippocampal slices, compared to control basal release (Fig. 6b). S18986-1 alone (3–1000 μM) also failed to induce [^{3}H]noradrenaline release in calcium-depleted buffer (data not shown). However, S18986-1 at 250 μM still retained the capacity to significantly enhance (*S*)-AMPA-mediated [^{3}H]noradrenaline release at (*S*)-AMPA concentrations of 100 μM (+80%) and 300 μM (+125%) (Fig. 6b).

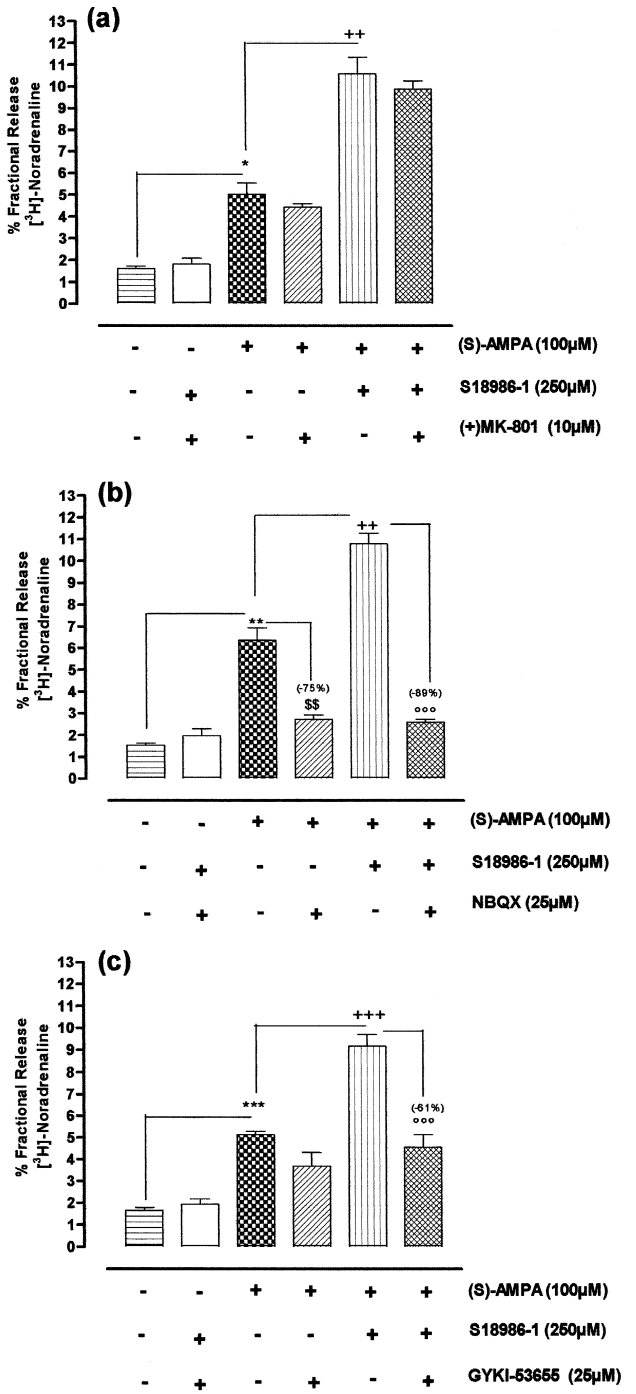

Fig. 5. Effect of NBQX, GYKI-53655, and (+)-MK-801 on S18986-1-stimulated [^{3}H]noradrenaline release. Prelabelled hippocampal slices were perfused at fraction #5 with either buffer alone, S18986-1 (250 μM) or S18986-1 (250 μM) + antagonist followed at fraction #6 by (*S*)-AMPA (100 μM). The different antagonists were (a) NBQX (25 μM) (b) GYKI-53655 (25 μM) or (c) (+)-MK-801 (10 μM). Data: mean ± S.E.M.; $n = 4$. Analysis: multiple Student's *t*-test corrected by Holm's adjustment (DMSO vs. (*S*)-AMPA; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$); ((*S*)-AMPA vs. (*S*)-AMPA + S18986-1; $^{++}P < 0.005$, $^{+++}P < 0.001$); ((*S*)-AMPA vs. (*S*)-AMPA + antagonist; $^{\$\$}P < 0.001$); ((*S*)-AMPA + S18986-1 vs. (*S*)-AMPA + S18986-1 + antagonist; $^{ooo}P < 0.001$).

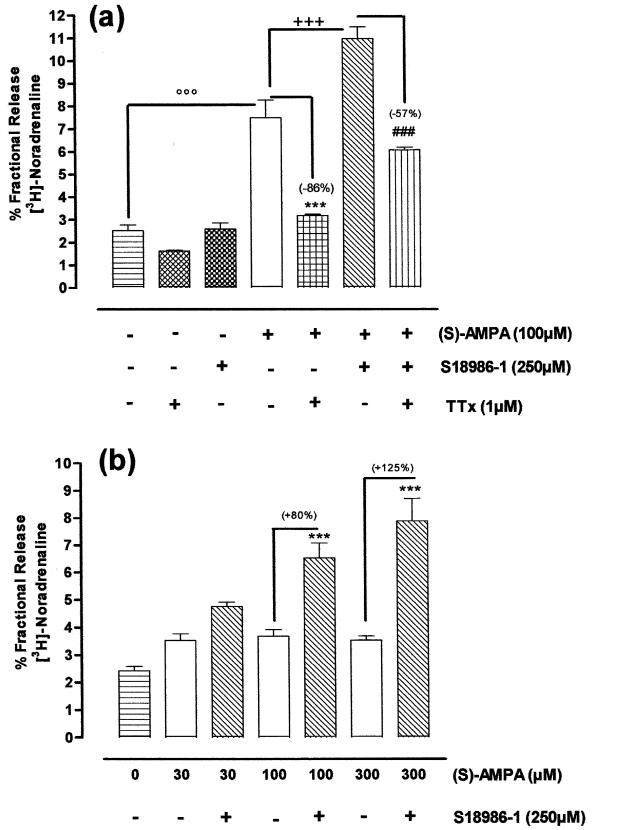

Fig. 6. Effect of Ca^{2+}-free buffer and tetrodotoxin on (*S*)-AMPA and S18986-1 stimulated [^{3}H]noradrenaline release in hippocampal slices. (a) Hippocampal slices were perfused with Krebs buffer in the absence or presence of S18986-1 (250 μM) and/or tetrodotoxin (1 μM) followed by (*S*)-AMPA (100 μM). Data: mean ± S.E.M.; $n = 4$. Analysis: one-way ANOVA with a complementary Newman–Keuls multiple comparison's test. (b) Hippocampal slices were perfused with Ca^{2+}-free buffer in the absence or presence of S18986-1 (250 μM) followed by (*S*)-AMPA at 30, 100, and 300 μM. Data: mean ± S.E.M.; $n = 4$. Analysis: two-way ANOVA with a complementary comparison's test (S18986-1 vs. S18986-1 + (*S*)-AMPA at 100 and 300 μM; $^{***}P < 0.001$).

3.3. Stereoselectivity of S18986-1-mediated enhancement of (S)-AMPA-induced [^{3}H]noradrenaline release

The stereoselectivity of S18986-1-mediated potentiation of (*S*)-AMPA-induced [^{3}H]noradrenaline release was established by comparison with its corresponding (*R*)-enantiomer, S19024-1 and racemic mixture S17951-1 (Fig. 7). As previously observed with S18986-1, neither S19024-1 nor S17951-1 alone were capable of stimulating [^{3}H]-NE release in hippocampal slices compared to basal release levels (Fig. 7). However, S18986-1 was significantly more potent (+164%) at stimulating (*S*)-AMPA-mediated [^{3}H]noradrenaline release than its corresponding (*R*)-enantiomer S19024-1 (+68%), relative to (*S*)-AMPA alone (Fig. 7a). Under similar conditions, S18986-1 was also more potent (+180%) than its racemic mixture S17951-1 (+124%) at enhancing (*S*)-AMPA-evoked [^{3}H]noradrenaline release (Fig. 7b).

3.4. Effect of cyclothiazide, and aniracetam on (S)-AMPA-induced [^{3}H]noradrenaline release in rat hippocampus

Cyclothiazide (0.3–100 μM), a prototypic AMPA receptor modulator failed to induce [^{3}H]noradrenaline release from rat hippocampal slices compared to basal DMSO control release levels (Fig. 8a). However, cyclothiazide induced a dose-dependent ($EC_{50} = 16.2$ μM (5.3–27.0)) increase in (*S*)-AMPA-evoked [^{3}H]noradrenaline release with maximal stimulation (+147%) observed at 100 μM cyclothiazide (Fig. 8a). On the other hand, the nootropic, aniracetam at concentrations of 0.3 and 1 mM failed to stimulate [^{3}H]noradrenaline release alone, or enhance (*S*)-

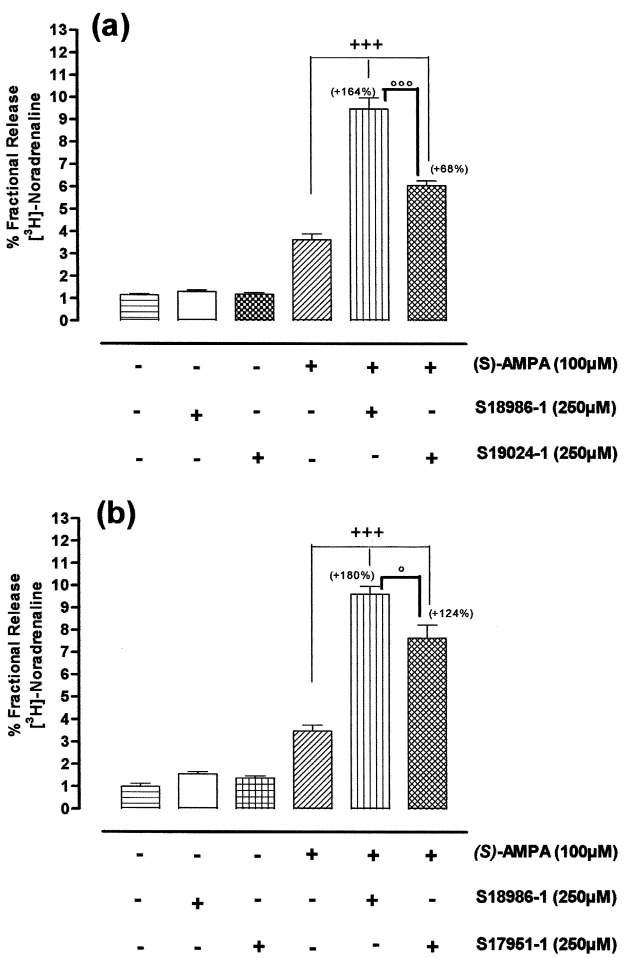

Fig. 7. Stereospecificity of S18986-1 stimulated [^{3}H]noradrenaline release. Prelabelled hippocampal slices were perfused with either buffer, S18986-1 (250 μM) or (a) S19024-1 (250 μM) or (b) S17951-1 (250 μM) in the absence and presence of (*S*)-AMPA (100 μM). Data: mean ± S.E.M.; $n = 4$. Analysis: two-way ANOVA ((*S*)-AMPA × treatment) with a complementary analysis of the interaction based on the contrast method (Drug × (*S*)-AMPA; $^{+++}P < 0.001$); and ((*S*)-AMPA × S18986-1 vs. (*S*)-AMPA × S19024 or S17951-1; $^{\circ}P < 0.05$; $^{\circ\circ\circ}P < 0.001$).

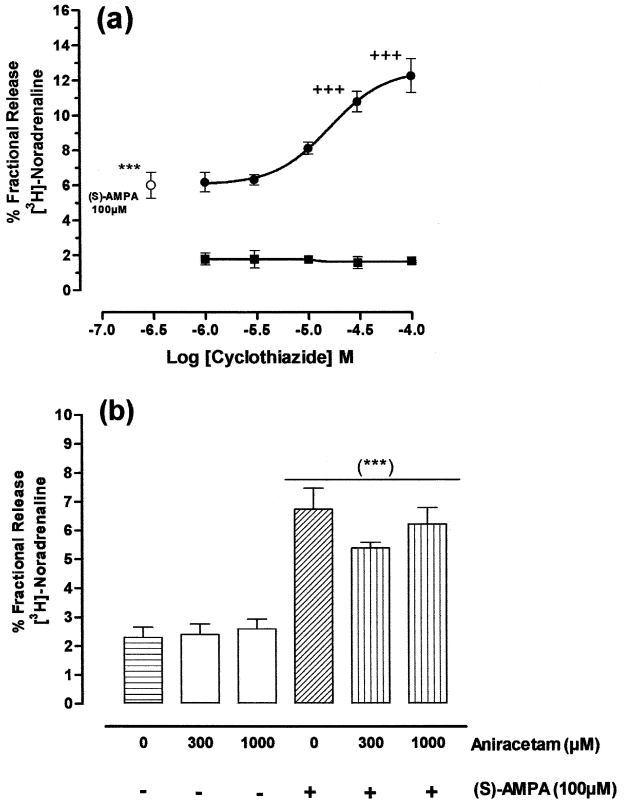

Fig. 8. Effect of cyclothiazide and aniracetam on (*S*)-AMPA-evoked [^{3}H]noradrenaline release. (a) Prelabelled hippocampal slices were perfused with different concentrations of cyclothiazide (0.3–100 μM) in the absence (-■-) or presence (-●-) of (*S*)-AMPA (100 μM). (*S*)-AMPA alone (-○-). Data: mean ± S.E.M.; $n = 4$. Analysis: one-way ANOVA with complementary Dunnett's test; (DMSO vs. cyclothiazide; $P = 0.915$ NS); DMSO vs. (*S*)-AMPA $^{+++}$ $P < 0.001$; ((*S*)-AMPA vs. (*S*)-AMPA + cyclothiazide; *** $P < 0.001$). (b) Slices were perfused with either buffer alone or aniracetam (0.3, 1 mM) at fraction #5 followed at fraction #6 by (*S*)-AMPA (100 μM). Data: mean ± S.E.M.; $n = 4$. Analysis: two-way ANOVA (aniracetam × AMPA, $P = 0.310$ NS); (aniracetam's effect $P > 0.05$) ((*S*)-AMPA's effect, *** $P < 0.001$).

AMPA (100 μM)-mediated release of [^{3}H]noradrenaline in rat hippocampal slices (Fig. 8b).

4. Discussion

In the present study, S18986-1, a positive modulator of AMPA receptors (Desos et al., 1996) markedly enhanced, in a concentration-dependent manner, (*S*)-AMPA-evoked [^{3}H]noradrenaline release in rat hippocampal and frontal cortex slices. Indeed, S18986-1 enhancement of AMPA-evoked noradrenaline release in rat brain slices, occurs in the same concentration range (30–300 μM) as S18986-1-mediated potentiation of AMPA-evoked currents in xenopus oocytes (Desos et al., 1996), as well as S18986-1-mediated increases in the amplitude of excitatory post-synaptic field potentials (e.p.s.f.p.s) in the CA1 region of hippocampal slices (Lepagnol et al., 1997). Moreover, in agreement with the stereoselectivity to potentiate AMPA-currents in Xenopus oocytes injected with rat cortex mRNA (Desos et al., 1996), S18986-1 was more potent at stimulating (*S*)-AMPA-mediated [^{3}H]noradrenaline release than its corresponding (*R*)-enantiomer and racemic mixture. S18986-1-mediated potentiation of [^{3}H]noradrenaline release was selective for the AMPA receptors subtype as no stimulation was observed with kainate under equivalent conditions. Furthermore, S18986-1 failed to enhance NMDA stimulated [^{3}H]noradrenaline release in Mg^{2+}-free Krebs buffer, and the NMDA antagonist (+)-MK-801 had no effect on S18986-1 stimulation of AMPA-induced [^{3}H]noradrenaline release, excluding any potential NMDA receptor-mediated mechanism.

Preliminary results (unpublished data) indicate potential differences in the estimated whole-brain levels of S18986-1 at doses producing cognitive-enhancing effects (Lebrun et al., 2000) compared to the effective concentrations in vitro (Desos et al., 1996; Lepagnol et al., 1997; this study). Nevertheless, several factors could explain these differences: firstly, S18986-1 is only active in the presence of (*S*)-AMPA in vitro, consequently, the activity of S18986-1 depends primarily on the state of activation of the AMPA receptor(s), and it is probable that activation of the AMPA receptor(s) in situ could be different from in vitro conditions. Secondly, the effective concentrations of S18986-1 gaining access to the synapse in hippocampal slices are likely to differ from the exogenously added concentrations, and thirdly, because of the heterogeneous distribution of AMPA receptors, the levels estimated in whole-brain could represent an underestimation of the effective concentration of S18986-1 compared to synaptic levels in hippocampal structures.

As previously demonstrated (Desai et al., 1994; Cowen and Beart, 1998; Desai et al., 1995; Pittaluga et al., 1997) cyclothiazide potently enhanced both AMPA- and kainate-evoked [^{3}H]noradrenaline release in rat hippocampal slices. However, although Pittaluga et al. (1999) observed a slight increase in AMPA-evoked [^{3}H]noradrenaline release with aniracetam in hippocampal slices and synaptosomes, no such effect was observed under the present experimental conditions. Consequently, the differential effects of S18986-1, cyclothiazide and aniracetam on AMPA-evoked [^{3}H]noradrenaline release in hippocampal slices supports the evidence suggesting distinct molecular effects for these ligands as regards the enhancement of AMPA receptor activation (Sekiguchi et al., 1998). Indeed, cyclothiazide appears to have greater selectivity towards the 'flip' isoform, whereas CX-516 and aniracetam appear to have a modest preference for 'flop' variants (Partin et al., 1994). It has also been suggested that although cyclothiazide blocks AMPA receptor desensitization with negligible effects on receptor deactivation, ampakines such as CX-516 appear to demonstrate an opposite pattern with preferential inhibition of AMPA receptor deactivation (Arai and Lynch, 1998). Based on the present results, S18986-1 may thus present a molecular effect on AMPA receptor

modulation, distinct from both cyclothiazide and aniracetam, in terms of its selectivity for the different AMPA-type glutamate receptor subunits (GluR1–GluR4) and/or towards the different 'flip' or 'flop' isoforms of the AMPA receptor.

(*S*)-AMPA-evoked [^{3}H]noradrenaline release in rat hippocampal slices was significantly inhibited in the presence of either tetrodotoxin and, in the absence of Ca^{2+} (Desai et al., 1994; Cowen and Beart, 1998; this study). However, S18986-1-mediated enhancement of AMPA-induced [^{3}H]noradrenaline release was only partially (-57%) inhibited with tetrodotoxin and furthermore S18986-1 still retained the capacity to potentiate [^{3}H]noradrenaline release in Ca^{2+}-free buffer. Indeed, previous reports have demonstrated that cyclothiazide-mediated enhancement of AMPA-induced [^{3}H]noradrenaline release was not completely ($\sim 60\%$) inhibited by tetrodotoxin treatment (Desai et al., 1994) suggesting that tetrodotoxin-insensitive mechanisms may also be implicated in the enhancement of AMPA receptor-mediated noradrenaline release with positive AMPA receptor modulators. The present results suggest a direct presynaptic control of noradrenaline release by AMPA-receptors (tetrodotoxin-insensitivity), as well alternative mechanisms involving interneurones (tetrodotoxin sensitivity). In addition, contaminating Ca^{2+} present in the tissue slices could partially explain the capacity of S18986-1 to retain its potentiating capacity in Ca^{2+}-free Krebs buffer. However, it is more likely, as observed in Fig. 2a, that S18986-1 is capable of enhancing AMPA-mediated [^{3}H]noradrenaline release at levels of (*S*)-AMPA which fail to induce a detectable release of [^{3}H]noradrenaline. Nevertheless, it cannot be excluded that spontaneous or alternative mechanisms of transmitter release implicating receptor-mediated potassium effluxes could also contribute to [^{3}H]noradrenaline release observed in the presence of (*S*)-AMPA. However, it is unlikely that S18986-1 potentiates (*S*)-AMPA evoked [^{3}H]noradrenaline release by simply modifying membrane depolarisation, secondary to receptor activation, as S18986-1 failed to enhance 4-aminopyridine-mediated release of [^{3}H]noradrenaline in rat hippocampus (unpublished data).

Noradrenaline exerts a positive role in both cognitive and attentional processes (Harley, 1991; Izquierdo et al., 1993) and noradrenergic terminals originating from neurones of the locus coeruleus, innervate many of the principal brain structures implicated in cognitive and attentional process including the hippocampus and frontal cortex. Previous reports have demonstrated that noradrenergic terminals present in rat hippocampus and frontal cortex contain AMPA-type receptors and that activation of the receptors induces the release of [^{3}H]noradrenaline (Pittaluga and Raiteri, 1992; Malva et al., 1994). Consequently, many of the cognition-enhancing actions of S18986-1 in rat (Lepagnol et al., 1997; Lebrun et al., 1998, 2000) could in part relate not only to the capacity of S18986-1 to increase the amplitude of e.p.s.f.p.s in rat hippocampal slices and to increase the duration of long-term potentiation responses in rat brain (Lepagnol et al., 1997), but also to its capacity to enhance AMPA receptor-mediated noradrenaline release in rat brain.

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