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A novel anti-epileptic agent, perampanel, selectively inhibits AMPA receptor-mediated synaptic transmission in the hippocampus

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ABSTRACT

Perampanel is a non-competitive AMPA receptor antagonist that is under development as an anti-epileptic therapy. Although it is known to reduce calcium flux mediated by AMPA receptors in cultured cortical neurons, there are no studies of its selectivity in synaptic transmission in more intact systems. In the present study using hippocampal slices, perampanel (0.01–10 μM) has been tested on pharmacologically isolated synaptic responses mediated by AMPA, NMDA or kainate receptors. Perampanel reduced AMPA receptor-mediated excitatory postsynaptic field potentials (f-EPSPs) with an IC_{50} of 0.23 μM and a full block at 3 μM. This compares with an IC_{50} of 7.8 μM for GYKI52466 on these responses. By contrast, perampanel at 10 μM had no effect on responses mediated by NMDA or kainate receptors, which were completely blocked by 30 μM D-AP5 and 10 μM NBQX respectively. The concentrations of perampanel required to reduce AMPA receptor-mediated responses are not dissimilar to those in plasma following anti-convulsant doses and are consistent with AMPA receptor antagonism being its primary mode of action.

1. Introduction

The ability to convey an anti-epileptic profile in humans can be seen with a wide variety of pharmacological agents (Lason et al., 2011; Rogawski, 2006) including sodium (see White et al. (2007)) and calcium (Weiergraber et al., 2010) channel blockers, potassium channel enhancers (Rogawski and Bazil, 2008), potentiators of GABA function (Czuczwar and Patsalos, 2001; Schousboe et al., 2011) and modulators of glutamate release (Moldrich et al., 2003; Sebastiao and Ribeiro, 2009). This list now extends into antagonists of glutamate receptors including convincing preclinical evidence for inhibition of N-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate receptors delivering anti-convulsant activity. Thus, agonists for NMDA (Chapman, 1998; Loscher, 1998), AMPA (Scharfman, 2007) and kainate (Lerma, 2006) receptors all induce seizure activity and their antagonists are effective in many seizure models (Meldrum and Rogawski, 2007; Rogawski, 2011). For example, the competitive, channel-blocking and glycine-site antagonists of NMDA receptors are anti-convulsant but have untoward side-effects, which limit their use (Kalia et al., 2008; Meldrum and Rogawski, 2007; Wasterlain and Chen, 2008). NMDA antagonism may, however, contribute to the anti-convulsant profile of felbamate (Kleckner et al., 1999) and possibly that of lacosamide (Beyreuther et al., 2007). AMPA receptor antagonists do not have such marked side effects and yet are very effective in animal models of epilepsy (Bleakman and Lodge, 1998; De Sarro et al., 2005; Hanada et al., 2011; Loscher and Honack, 1994; Rogawski, 2011; Yamaguchi et al., 1993). AMPA receptor antagonists may be particularly beneficial in combination with other treatments (Jonker et al., 2007) and have been shown to be effective in add-on studies in man with a limited side effect profile (Chappell et al., 2002; Langan et al., 2003). AMPA receptor antagonism contributes to the anti-convulsant effects of lamotrigine (Lee et al., 2008). Kainic acid is the prototypical amino acid convulsant (Jane et al., 2009; Nadler et al., 1981; Vincent and Mulle, 2009) and kainate antagonists particularly of the GluK1 subtype have anti-epileptic potential (Jane et al., 2009; Matute, 2010; Smolders et al., 2002). It seems likely that GluK1 antagonism underlies some of the anticonvulsant effects of topiramate (Braga et al., 2009). Despite the rationale and the preclinical evidence, no compound developed as a glutamate receptor antagonist has yet become a clinically accepted therapy for the treatment of epilepsy.

It is therefore of considerable interest that perampanel, a compound thought to be a non-competitive antagonist of AMPA receptors, is under development for epilepsy treatment (Hanada et al., 2011). Perampanel has been shown to reduce AMPA-, but not NMDA-evoked calcium signals in rat cortical neurones in culture (Hanada et al., 2011). However, no studies on excitatory synaptic transmission in mammalian brain have been reported for this compound. We therefore investigated the actions of perampanel on

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synaptic responses known to be mediated selectively by either NMDA, AMPA or kainate receptors. We report here that perampanel selectively reduces AMPA receptor-mediated synaptic transmission relative to that mediated by NMDA and kainate receptors at concentrations likely to be achieved in the brain at clinically relevant doses.

2. Materials and methods

2.1. Slice preparation and electrophysiological recordings

Experiments were performed according UK Scientific Procedures Act, 1986 and EU guidelines for animal care. Transverse hippocampal slices from adult male Wistar rats (340–390 g) were cut, prepared and pre-incubated with solutions as described previously for CA1 recording (Volianskis and Jensen, 2003) and for CA3 recording (Schmitz et al., 2001). For CA1 recording, slices were kept submerged at ≈31 °C and superfused at a rate of 3 ml/min with an artificial cerebrospinal fluid (aCSF) solution (in mM: 124 NaCl, 3.5 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 2 $CaCl_2$, 2 $MgSO_4$ and 10 glucose), which was saturated with 95% O_2 and 5% CO_2. For CA3 recording, slices were transferred to an interface chamber and superfused with aCSF (as above except 3 KCl and 1 $MgSO_4$).

AMPA receptor-mediated field excitatory postsynaptic potentials (f-EPSPs) were recorded in the CA1-B area of stratum radiatum using glass electrodes filled with saline solution in response to electrical stimulation (100 μs duration) of the Schaffer collaterals. f-EPSPs were evoked at a frequency of 0.067 Hz and the stimulation current was fixed to three times the threshold for evoking the f-EPSPs. After a stable period of at least 30 min (baseline) pharmacological agents were applied to examine their effects on AMPA receptor-mediated responses.

To study NMDA receptor-mediated f-EPSPs, first a stable baseline period of AMPA receptor-mediated responses was achieved and then 10 μM NBQX was added to the perfusate to block the AMPA receptor-mediated f-EPSP. To obtain an NMDA receptor-mediated f-EPSP, the divalent cation ratio in the saline solution was increased to 3 mM $CaCl_2$, 1 mM $MgSO_4$ and the stimulation intensity was increased 2–5 times. 30 μM D-2-amino-5-phosphono-pentanoate (D-AP5) was applied at the end of the experiments on NMDA receptor-mediated f-EPSPs to confirm pharmacological isolation of the responses.

To study kainate receptor-mediated mossy fibre (MF) responses at CA3 synapses, electrical stimulation was applied to granule cell axons close to the stratum granulosum of the dentate gyrus; the evoked f-EPSPs were recorded in CA3 stratum lucidum using the WinLTP software (Anderson and Collingridge, 2007; http://www.winltp.com). MF f-EPSPs were identified, in the presence of 50 μMD-AP5, 100 μM GYKI52466 and 50 μM picrotoxin, by characteristic facilitation during high frequency stimulation (Bortolotto et al., 1999; Lauri et al., 2001; Nicoll and Schmitz, 2005).The kainate receptor-mediated synaptic responses were triggered by 2–4 trains of 5 stimuli at 50 Hz delivered at 2 min intervals.

2.2. Analysesof electrophysiological recordings

To quantify the effects of compounds on AMPA receptor-mediated synaptic transmission the initial (≈0.8 ms) slopes of AMPA f-EPSPs were measured and normalised to baseline. The efficacy of NMDA receptor-mediated synaptic transmission was assessed by subtracting the mean waveform after application of 30 μM D-AP5 from each preceding waveform in the individual experiments, measuring the amplitudes of NMDA f-EPSPs and normalising them to baseline. As an index of kainate receptor-mediated activity in the presence of D-AP5, GYKI52466 and picrotoxin, the size of the fifth population spike in the train was measured before and after application of compounds.

2.3. Chemicals

D-2-amino-5-phosphono-pentanoate (D-AP5), 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline-2,3-dione (NBQX), 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine (GYKI 52466), picrotoxin (PTX), tetrodotoxin (TTX) and cyclothiazide (CTZ) were acquired from Ascent. Perampanel was a gift from T. Hanada, Eisai Co., Ltd. Compounds were prepared as stock solutions, stored frozen and added to the perfusing saline as indicated in results. All other chemicals and salts were from Fisher Scientific or Sigma.

2.4. Data presentation and statistical analyses

Data are presented both as single experiments and as mean values of experimental groups (± S.E.M). Student's (two-tailed) *t* tests were used for either within (paired) or between (unpaired) group comparisons. One-way ANOVA followed by Newman-Keuls Multiple Comparison Test (NKMCT) was used in cases of multiple comparisons. F test was used for comparison of concentration response curves (Prism 5, GraphPad). Significant differences were set at $P < 0.05$.

3. Results

3.1. Perampanel is a potent antagonist of AMPA receptors

The effects of perampanel on AMPA receptor-mediated synaptic transmission were investigated by recording f-EPSPs in the stratum radiatum of the CA1 area in hippocampal slices in response to electrical stimulation of the Schaffer collaterals. Application of 0.3 μM perampanel reduced the slopes of AMPA receptor-mediated f-EPSPs to about half of their initial value, in a manner which was not affected by 100 μM cyclothiazide (CTZ, Fig. 1A). This result indicates that the binding site for perampanel and CTZ is not the same. Notably, CTZ prolonged the decay of AMPA f-EPSPs (see representative f-EPSPs in Fig. 1A). The residual AMPA receptor-mediated response was blocked by 10 μM NBQX (Fig. 1A). The equilibration time for the effect of 0.3 μM perampanel on f-EPSPs was relatively long, taking approximately 1 h to reach a steady state. However, much shorter equilibration times were reached when using 1 and 10 μM of perampanel (Fig. 1B and C, closed circles) and the effects of perampanel on AMPA receptor-mediated f-EPSPs were independent of stimulation during wash-in (Fig. 1B, open circles) and during wash-out (Fig. 1C, open circles) of the compound. These results suggest that the actions of perampanel on AMPA receptor-mediated synaptic responses are not use-dependent.

The potency of perampanel was quantified further in a series of experiments (Fig. 2A), which showed that perampanel inhibited AMPA receptor-mediated f-EPSPs with an IC_{50} of 0.23 μM (Fig. 2C) with a complete block at 3 μM. Perampanel was therefore 34-fold more potent than the prototypical non-competitive AMPA antagonist GYKI 52466, which blocked AMPA receptor-mediated f-EPSPs with an IC_{50} of 7.8 μM (Fig. 2B and C).

3.2. Perampanel has no effect on NMDA receptors

The effects of perampanel on NMDA receptor mediated synaptic transmission were investigated similarly to those of AMPA, albeit after blocking AMPA receptor-mediated responses with 10 μM NBQX, which completely obliterates AMPA receptor-mediated f-EPSPs. After a complete block of AMPA receptor-mediated f-EPSP,

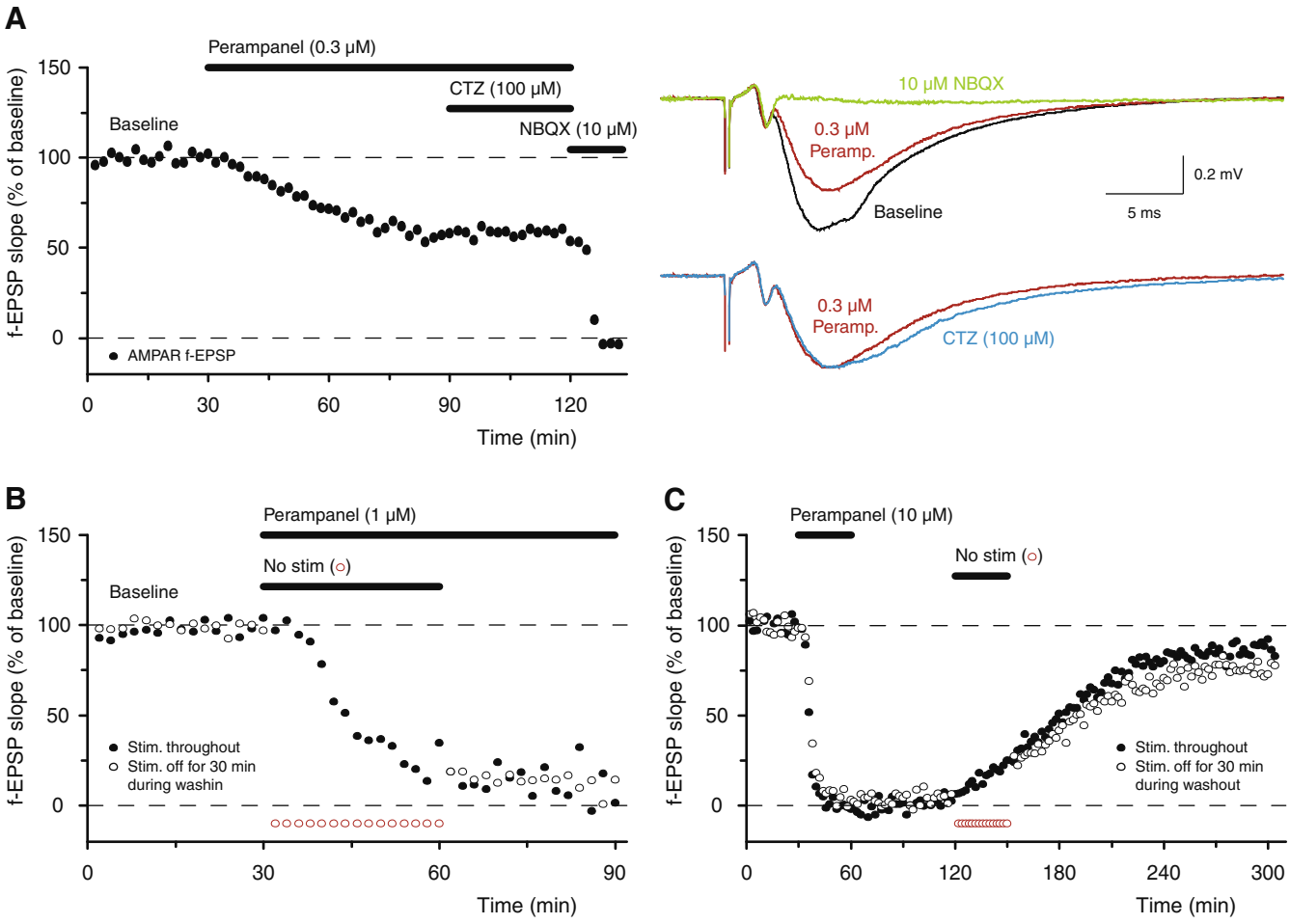

Fig. 1. Effects of perampanel on AMPA receptor-mediated transmission are not use dependent. (A) Application of 0.3 μM perampanel resulted in a reduction of AMPA receptor-mediated f-EPSPs (filled circles, application of compounds in this and subsequent figures are indicated by thick lines above the trace). The waveforms from this experiment (inset to the right), visualise the effects of 0.3 μM perampanel (red) and 10 μM NBQX (green) on AMPA receptor-mediated control responses (black). This experiment has been repeated 4 times. Note that application of 100 μM cyclothiazide (CTZ, blue), in the presence of perampanel, had no effect on the slope of AMPA receptor-mediated f-EPSPs, but prolonged their decay time. Calibration bar is shown in the inset. (B) Inhibition by perampanel of AMPA receptor-mediated f-EPSPs did not require stimulation during the wash-in (open vs. closed circles, as indicated) of the compound (n = 2 and 3, with and without the delay respectively). (C) Recovery during wash-out of perampanel was also independent of the stimulation (n = 2 and 5, with and without the delay respectively).

the stimulation was increased to evoke an NMDA receptor-mediated response. At a concentration of 10 μM, perampanel had no effect on NMDA receptor-mediated f-EPSPs (Fig. 3A) whereas application of 1 μM and 30 μM of the prototypical competitive NMDA antagonist D-AP5 reduced NMDA receptor-mediated f-EPSPs by nearly 50% and 100% respectively. Thus, at a concentration of 10 μM perampanel is selective between AMPA and NMDA receptors; it blocks AMPA receptor-mediated responses completely and has no effect on NMDA receptor-mediated responses.

3.3. Perampanel has no effect on kainate receptors

After obtaining stable and facilitating AMPA receptor-mediated responses in CA3 to mossy fibre stimulation in the dentate gyrus, 100 μM GYKI52466, 50 μM D-AP5 and 50 μM picrotoxin (PTX) were used to block AMPA, NMDA and GABA-A receptor-mediated responses respectively. A train of 5 stimuli at 50 Hz was used to activate kainate receptors, which resulted in a stimulus-dependent facilitation of the evoked response (Fig 4A; see (Bortolotto et al., 1999; Lauri et al., 2001; Vignes and Collingridge, 1997). The kainate receptor-mediated f-EPSPs and the facilitation of the population spike were blocked by NBQX 10 μM, (Fig 4A; upper panel), presumably due to its activity at kainate receptors (Bortolotto et al., 2003). Perampanel (10 μM), by contrast, was without an effect on either the f-EPSPs or the facilitation (Fig 4A; lower panel). The size of the fifth population spike in the 50 Hz train, after superfusion of the compound, is expressed as a percentage of that in control aCSF, which contained GYKI, AP5 and PTX (Fig 4B). The effect of perampanel is insignificant compared to control whereas there is a significant reduction with NBQX ($P < 0.05$). Thus, at a concentration of 10 μM, perampanel is highly selective between responses mediated by AMPA receptors and those mediated by kainate receptors, fully blocking the former and with no significant effect on the latter.

4. Discussion

Perampanel reduced AMPA receptor-mediated f-EPSPs in a concentration-dependent manner and with at least a 100-fold separation over synaptic responses mediated by NMDA and kainate receptors. For example, 0.1 μM perampanel produced a significant reduction of AMPA receptor mediated f-EPSP (Fig. 2A) whereas 10 μM was without an effect on NMDA- (Fig. 3) and kainate- (Fig. 4) receptor-mediated synaptic events in this preparation. Of course, we cannot discount an effect on other neural pathways where the subunit compositions of the receptors responsible may

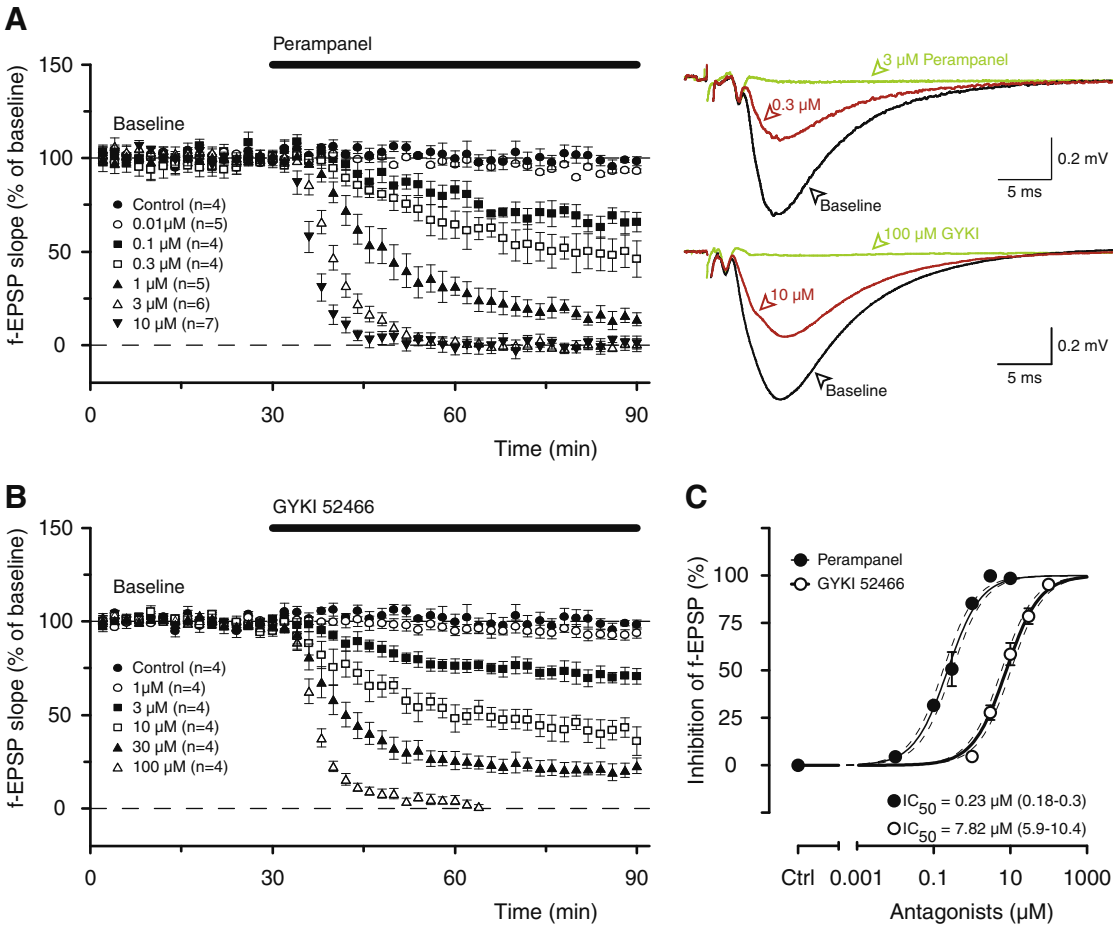

Fig. 2. Perampanel is more potent than GYKI 52466. (A) A series of experiments showing concentration-dependent inhibition of AMPA receptor-mediated f-EPSPs by perampanel (4–7 slices per group, mean ± S.E.M.). 0.01 μM perampanel (open circles) had no significant effect on AMPA receptor-mediated f-EPSPs when compared to the control (filled circles, $P > 0.05$, NKMCT), whereas application 0.1 μM (or any higher concentration) reduced f-EPSPs significantly (filled squares, $P < 0.001$, NKMCT). Application of 3 μM perampanel resulted in a complete inhibition of AMPA receptor-mediated synaptic transmission. Inset shows representative waveforms from two individual experiments using perampanel (0.3 μM; red) and GYKI52466 (GYKI; 10 μM; red). The green traces for 3 μM perampanel and 100 μM GYKI are from separate experiments and are shown to illustrate their complete inhibition of the AMPA receptor mediated responses. (B) Effects of GYKI52466 on AMPA receptor-mediated f-EPSPs (4 slices per group). (C) Perampanel (IC_{50} = 0.23 μM) is 34-times more potent than GYKI52466 (IC_{50} = 7.82 μM, $P < 0.001$, confidence intervals for the IC_{50} values are given in parentheses).

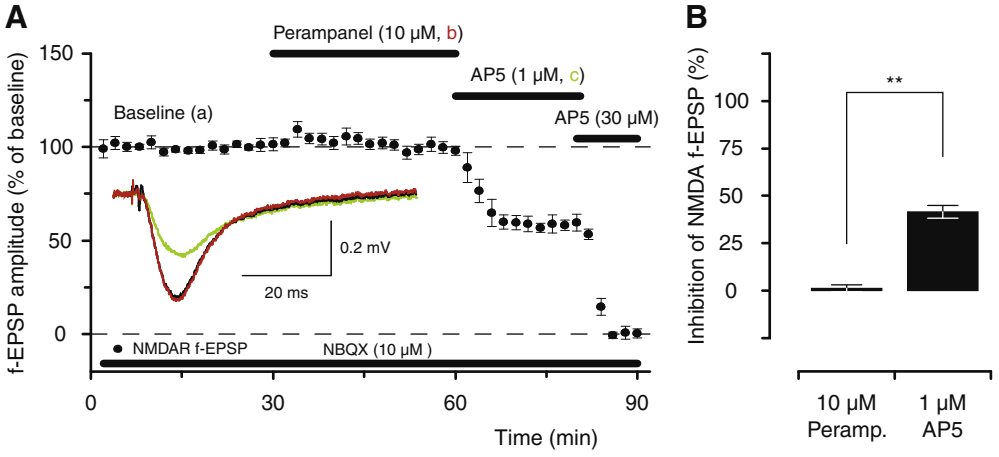

Fig. 3. Perampanel has no effect on NMDA receptor-mediated f-EPSPs (A) a group of experiments showing that 10 μM perampanel has no effect on NMDA receptor-mediated f-EPSP (98.9 ± 1.9% of baseline response) whereas 1 μM D-AP5 provides a significant inhibition of NMDA receptor-mediated f-EPSP (58.5 ± 3.4%) and 30 μM D-AP5 provides a full block ($n = 4$). (B) Effects of 10 μM perampanel and 1 μM D-AP5 differed significantly ($P < 0.01$, paired t-test).

be different. The AMPA receptor antagonist effect of perampanel was not use-dependent in its onset or recovery, which is consistent with perampanel not acting as an open channel blocker. The lack of effect of cyclothiazide also suggests that perampanel does not increase the rate of desensitization and the lack of obvious change in kinetics of the reduced AMPA receptor-mediated EPSPs agrees

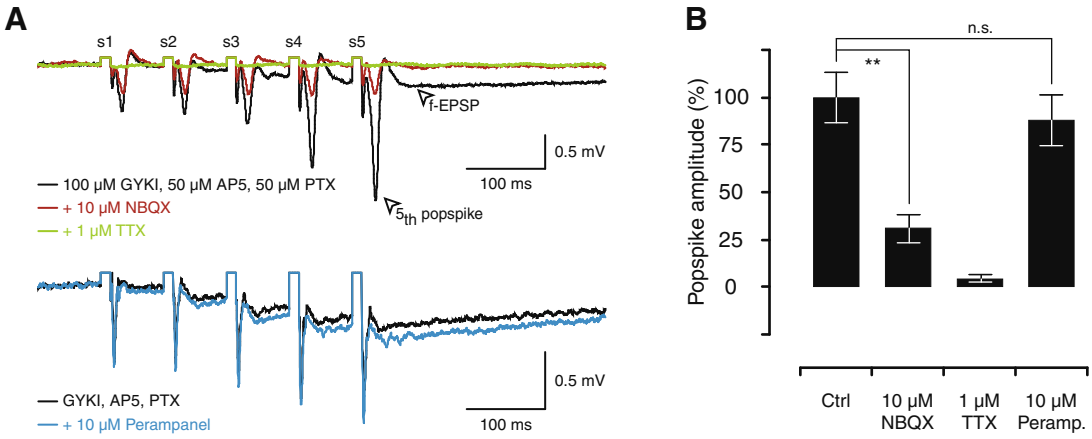

Fig. 4. Perampanel has no effect on kainate receptor-mediated responses (A) Top panel shows representative waveforms from an experiment in which activation of kainate receptors induced facilitation of the population spike (black), which was blocked by 10 µM NBQX (red). The residual, non-synaptic component of the response was blocked by 1 µM TTX (green). Bottom panel depicts that perampanel (10 µM, blue) had no effect on facilitation of mossy fibre responses. (B) For presentation purposes the control data from the two sets of experiments that are depicted in panel A were pooled ($n = 8$, Ctrl). Application of perampanel did not significantly affect the size of the fifth population spike when compared to the control (88.0 ± 13.4, $n = 4$, $P = 0.4$, paired t test) whereas NBQX abolished the fifth population spike evoked through activation of kainate receptors (30.9 ± 7.6, $n = 4$, $P = 0.03$, paired t test). The residual component of the response in NBQX was blocked by TTX (4.4 ± 2.0).

with this interpretation, although such a conclusion has to be further confirmed by intracellular experiments. Analysis of f-EPSPs with respect to the possible competitive or non-competitive nature of the antagonism by perampanel is not possible, owing to the recruitment of more synapses with increasing stimulus strength rather than changes in postsynaptic receptor occupancy. However, the published data (Hanada et al., 2011) are very convincing in showing that the antagonism of AMPA receptors by peramapanel is like that of the 2,3-bezodiazepine, GYKI52466 (Bleakman and Lodge, 1998), non-competitive in its nature and not as result of open channel block. The present data are consistent with that conclusion.

The reported IC_{50} of approximately 100 nM (CI: 45–150 nM; versus 2 µM AMPA) for perampanel in a calcium flux assay in cortical neuronal cultures (Hanada et al., 2011) is similar to that of the 230 nM found here versus the AMPA receptor-mediated f-EPSPs in the Schaffer collateral-CA1 pathway in rat hippocampal slices. Total and free plasma concentrations in the region of 1190–1670 nM and 155–217 nM perampanel, respectively, are achieved following oral administration of 5 mg/kg p.o. to rats, which is a protective dose in the rat kindling model of epilepsy, and perampanel equilibrates well across the blood–brain barrier (Hanada et al., 2011); Hanada, personal communication). It therefore seems highly likely that the brain concentrations achieved following such dosing and responsible for the anti-seizure activity would produce AMPA receptor antagonism resulting in reduced synaptic excitation. Indeed, our data would suggest that at therapeutic concentrations perampanel is reducing, but not abolishing, AMPA-receptor-mediated synaptic transmission in the CNS, which appears sufficient to provide anticonvulsant activity without producing untoward effects.

Selective non-competitive AMPA receptor antagonism has been associated with a low side effect profile (Chappell et al., 2002; Langan et al., 2003; Rogawski, 2011) and the lack of effect of perampanel on synaptic NMDA receptor-mediated responses suggests that psychotomimetic issues are less likely to arise (Kalia et al., 2008; Meldrum and Rogawski, 2007; Wasterlain and Chen, 2008). Recent reports have indicated that perampanel, at doses of up to 12 mg/day, is tolerated in humans (Krauss et al., 2011a), and from 4 mg/day is effective as an add-on anti-epileptic treatment in double blind trials in patients with refractory partial onset seizures (French et al., 2011a; French et al., 2011b; Krauss et al., 2011b). In a recent report, the 4 and 12 mg doses were found to give mean plasma concentrations of about 400 and 1000 nM respectively (Laurenza et al., 2011). We therefore conclude that anti-epileptic effects of perampanel are likely to be mediated through selective antagonism of AMPA receptors.

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