Effect of FK960, a Putative Cognitive Enhancer, on Synaptic Transmission in CA1 Neurons of Rat Hippocampus

JOSEPH P. HODGKISS and JOHN S. KELLY
Fujisawa Institute of Neuroscience, Department of Neuroscience, University of Edinburgh, Edinburgh, United Kingdom
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ABSTRACT
The action of FK960 [N-(4-acetyl-1-piperazinyl)-p-fluorobenzamide monohydrate], a novel cognitive enhancer, on excitatory synaptic transmission in the hippocampus was investigated. Excitatory postsynaptic potentials (EPSPs) and currents (EPSCs) were recorded intracellularly from CA1 neurons in rat hippocampus using the “blind patch” variant of whole-cell recording. FK960 (100 nM) significantly increased the amplitude of the EPSP, which was unchanged when changeover was made to control artificial cerebrospinal fluid (aCSF). FK960 had no significant action on membrane potential, input resistance, or the early GABAergic inhibitory postsynaptic current. The decay phase of the excitatory postsynaptic current was not significantly altered by exposure to FK960, indicating that the properties of desensitization and/or deactivation were unchanged and suggesting that the action of FK960 was unlikely to be the result of changes in the properties of the postsynaptic (S)-α-amino-3-hydroxy-5-methyl-4-isoxazoloproprionic acid (AMPA) receptors. The quantal content of the EPSP (1/CV²) increased after exposure to FK960 but not to control aCSF. Methyllycaconitine or α-bungarotoxin blocked the modulatory action of FK960 on the EPSP, and the finding that these α7-nicotinic acetylcholine receptor (α7AChR) antagonists were effective raises the possibility that FK960 up-regulates the contribution of acetylcholine to synaptic efficacy in the hippocampus. It is concluded that FK960 increases the quantal release of glutamate from Schaffer collateral-commissural nerve terminals in area CA1 of the hippocampus either by changing the ambient level of acetylcholine or by positively modulating the activity of α7AChRs located on glutamatergic nerve terminals.

During the neuronal attrition, which underlies Alzheimer’s disease, there is a progressive loss of neuronal projections to the cortex and hippocampus. The loss of cholinergic projections is marked and has been the subject of many studies (see Kasa et al., 1997 for references). At present, no cure is in prospect and palliative treatment is aimed at up-regulating the function of the reduced pool of available neurons. In the brain, as in the periphery, it is possible to enhance synaptic transmission either presynaptically, by increasing the amount of transmitter released, or postsynaptically, by modulating the behavior of the postsynaptic receptors or reducing the breakdown of transmitter. In Alzheimer’s disease, there is a loss of choline acetyltransferase, the enzyme involved in acetylcholine synthesis; thus treatments have concentrated on up-regulating cholinergic transmission in the brain, particularly by inhibition of cholinesterase (Francis et al., 1999).

We report here studies of FK960 on glutamatergic transmission in CA1 neurons in rat hippocampal slices. FK960 has been shown to reverse scopolamine-induced cognitive deficits in rats in vivo (Yamazaki et al., 1996), to increase the magnitude of long-term potentiation in guinea pig hippocampus in vitro (Matsuoka and Satoh, 1998), and improve visual recognition memory in primates (Matsuoka and Aigner, 1997). These studies implicated somatostatinergic, cholinergic, and serotonergic systems but did not, however, indicate whether FK960 acts pre- or postsynaptically nor did they rule out the involvement of other transmitter systems, such as glutamate or GABA. More recently, it has been shown that FK960 increases the amplitude of the unpotentiated population spike in rat hippocampus (Matsuyama et al., 2000). A role for glutamatergic transmission in memory has been advanced by studies with AMPAkines, such as BD-P12 and related compounds that enhanced memory
(Granger et al., 1996; Lynch et al., 1996), increased the degree and duration of long-term potentiation (Staubli et al., 1994), and the amplitude and duration of the field EPSP in the hippocampus by changing the characteristics of AMPA receptor desensitization and/or deactivation (Arai et al., 1996a,b; Sirvio et al., 1996; Arai and Lynch, 1998). The present study shows FK960 to enhance transmitter release at AMPAergic synapses on CA1 neurons in the hippocampus and raises the possibility that FK960 acts on the nerve terminal to increase transmitter release. The effect of block of α7nAChRs by methyllycaconitine or α-bungarotoxin on the action of FK960 was also examined to determine the involvement, if any, of this subtype of acetylcholine receptor.

Materials and Methods

Male rats (Sprague-Dawley, 50–100 g; Charles River, Montreal, Quebec) were decapitated, the brain was removed, and placed in well oxygenated ACSF at 4°C, which had the following composition in mM: NaCl, 126; KCl, 2.75; NaHCO3, 26; Na2HPO4, 1.25; d-glucose, 10; MgSO4, 1.8; and CaCl2, 2.5. The brain was then placed ventral side down on the trimming block, and four cuts were made to isolate the hippocampus. The resulting block of tissue was attached, using a few drops of cyanoacrylne adhesive, to the platform of the tissue-slicing apparatus (Vibroslice, Campden Instruments, Loughborough, UK) and 450-μm-thick transverse slices cut. The slices were placed in well oxygenated ACSF at room temperature and allowed to equilibrate for at least 1 h. In experiments in which α-bungarotoxin was studied, the holding chamber ACSF also contained 500 nM α-bungarotoxin. Slices were transferred to the recording chamber, also at room temperature, from 1 to 7 h after being cut. The flow rate was 2.5 to 3.0 ml · min−1 and was monitored throughout the experiment; data were not accepted from cells in which the flow rate fell below 2.5 ml · min−1. The ACSF was gassed with 95% O2/5% CO2. A single razor cut was made to isolate CA3 from the CA1 subfield. Movement of the slice was prevented by platinum weights. EPSPs were evoked using stainless steel wire stimulating electrodes, placed on the stratum radiatum.

Patch electrodes, pulled on a Narishige P83 vertical puller, had resistances of 5 to 8 MΩ when filled with the following solution; potassium gluconate, 120; KCl, 10; NaCl, 5; EGTA, 10, HEPES, 10; MgCl2, 2, CaCl2, 1; NaATP, 2; and NaGTP, 1 (brought to pH 7.3 with KOH). In experiments in which FK960 was compared with BDP-12 [also listed as CX516, Cortex Pharmaceuticals (San Leandro, CA) and synthesized by Fujisawa Pharmaceutical Co. (Osaka, Japan) as FR212436] on the EPSC, 4 mM QX314 (Tocris) and 500 μM D-AP5 (Tocris). Methyllycaconitine (Sigma, St. Louis, MO, and Calbiochem, San Diego, CA) dissolved in distilled water to give stock solutions of 100 μM, which were serially diluted to achieve adequate block of GABA3 and NMDA receptors. Input resistance of the neuron was monitored at the start and at times throughout the experiments by injecting hyperpolarizing current pulses (intensity 0.05 nA, duration 400 ms). When the action of FK960 on the EPSP was examined, stimulus intensity was adjusted so that EPSPs with a mean amplitude of about 2 mV were evoked. This ensured that 1) only a relatively small number of axons were excited and 2) the stimulus evoked-EPSPs were subthreshold for action potential discharge under control and test conditions. EPSPs were evoked at 0.25 Hz, and after 5 min the bath solution was changed to one of the following: control ACSF; methyllycaconitine (10 or 100 nM) in control ACSF; or α-bungarotoxin (300 nM) in control ACSF for 15 min. In the continued presence of the pretreatment drug, the preparation was then exposed either to an ACSF to which was added either FK960 (at a concentration of 100 nM) or control ACSF containing only the pretreatment drug. EPSPs were collected for at least a further 24 min. EPSPs were recorded continuously on tape (DAT recorder model DTR-1205, Biologic Science Instruments, Cliax, France) and sampled off-line, digitized at 2.5 to 5.0 kHz and filtered at 1 kHz using an 8-pole Bessel filter.

EPSP amplitudes were measured using either patch and voltage clamp (version 6.36) or Signal (version 1.8) software (CED, Cambridge Electronic Design, Cambridge, UK). Two cursors were placed on each record; one cursor was positioned on the baseline before the stimulus artifact and a second on the peak of the EPSP. The EPSP amplitude data were confirmed by measuring the slope of the rising phase of the intracellularly recorded EPSP by placing cursors at 10% and 50% of peak amplitude, and measuring the slope between these two points using a program written in Signal (CED, Cambridge). In a separate series of experiments, CA1 neurons were voltage clamped at −70 mV in continuous mode (Axoclamp 2A, Axon Instruments), and EPSCs were evoked; series resistance was checked by applying 5 or 10 mV hyperpolarizing voltage pulses at regular intervals. The time constant of the decay phase of the EPSC was determined by fitting a single exponential (patch and voltage clamp software, version 6.36, Cambridge). The GABAergic IPSC, evoked at a rate of 0.1 Hz, was also studied after block of AMPA and NMDA receptors with, respectively, 15 μM CNQX (Tocris) and 50 μM D-AP5 (Tocris).

Quantal content (mCV), a measure of presynaptic function, was determined by the “variance” method (Del Castillo and Katz, 1954; Martin, 1977), which is based on the idea that trial-to-trial variation in EPSP amplitude reflects the probabilistic organization of the quantal release mechanism. Quantal content (mCV) was determined from the 100 EPSPs recorded prior to changeover to either control ACSF or FK960 and from the same number measured after 21-min exposure to either FK960 or control ACSF. EPSP amplitude was measured as previously detailed, except that two cursors were placed on the baseline before the stimulus artifact to measure the amplitude and variance of the noise and a third placed on the peak of the EPSP. The mean (E) and standard deviation (s) of the series of EPSPs were determined. The assumption made was that release at synapses between Schaffer collateral-commissural axons and CA1 neurons, under the conditions of our experiments, conforms to Poisson’s Law. Then (Martin, 1977)

\[ m_{CV} = 1/CV^2 \]

where

\[ CV = \sigma_E / \sigma^2 \]

after correction for the variance of the noise (\( \sigma^2 = \sigma_n^2 - \sigma_n^2 \)), where \( \sigma_n^2 \) is EPSP variance and \( \sigma_n^2 \) the variance of the noise. The calculation did not include correction for the variation in quantal size. Thus, CV as calculated above will be somewhat greater than the CV of the quantal distribution. No corrections were made for nonlinear summation of the EPSPs. Quantum size, a measure of the postsynaptic effect of a quantum of transmitter, was determined from the ratio of EPSP amplitude to quantal content. To determine the frequency of
spontaneous EPSPs, the 1 s following each stimulus was omitted from the analysis; thus, only events in the 3 s preceding each stimulus were included.

The experiments were carried out pseudorandomly using a sequence generated using the RAND function in Microsoft Excel. EPSP amplitudes and slopes were measured before exposure to FK960 (t = −1 min 40 s, hereafter simplified to −1 min) and after exposure (t = +21 min 40 s, simplified to 21 min) to either FK960 in the pretreatment solution or the pretreatment solution to which no FK960 was added. The 21 min 40 s time period represents the midpoint of the sampling period in which 50 consecutive EPSPs were measured following a 20-min exposure to 100 nM FK960. A one-way analysis of variance of the changes in EPSP amplitude was performed using a multiple comparison procedure (SigmaStat version 2.0, Jandel, San Rafael, CA). For other comparisons, a t test or paired t test was performed as appropriate.

**Results**

The concentrations of FK960 to investigate were determined in a preliminary series of experiments in which doses of 0 nM (n = 6 neurons), 50 nM (n = 5), 100 nM (n = 5), and 200 nM FK960 (n = 5) were examined for their effect on the slope of the EPSP. The greatest increase in slope was seen with 100 nM FK960 (Fig. 1). The increase in EPSP slope by 55 ± 13% (S.E.M., n = 5) was significantly greater than the 11 ± 10% increase seen in control aCSF (P = 0.04, one-way ANOVA). None of the other groups differed significantly from control. Matsuoka and Satoh (1998) also found 100 nM FK960 to increase significantly the magnitude of long-term potentiation in guinea pig hippocampus. Consequently, in the experiments reported here, 100 nM FK960 was used.

**Effect of FK960 on Membrane Potential and Input Resistance.** The effect of 100 nM FK960 on the passive membrane properties of CA1 neurons was examined and found to have no significant action on either the resting membrane potential of −60.3 ± 0.9 mV (n = 11) in control aCSF and −59.4 ± 0.7 mV in FK960 (P = 0.13, paired t test), or input resistance; 175.3 ± 23.8 MΩ (n = 11) in control aCSF and 164.2 ± 221.7 MΩ in FK960 (P = 0.09, paired t test). The average exposure time was 30 ± 2 min.

**Effect of FK960 on the EPSP.** Intracellular recordings from CA1 neurons were usually maintained for more than 1 h. After rupturing the cell membrane to go into whole-cell mode, and confirming the stability of the recording, the bathing solution was exchanged for one containing 50 μM d-AP5, 100 μM picrotoxin, and 10 μM bicuculline (control aCSF) to block NMDA and GABAA receptors. In Fig. 2, records from a typical experiment show the EPSP in a CA1 neuron obtained 15 min after exposure to control aCSF. Stimulation of the stratum radiatum resulted in EPSPs that fluctuated in amplitude; in this experiment, the range was from 0.2 mV to 5.8 mV (Fig. 2C). The averaged record of 50 consecutive EPSPs in control aCSF is shown in Fig. 2A. The slope of the rising phase, determined from 10% to 50% of peak amplitude, was...
and variance of 100 EPSP amplitudes recorded prior to exposure to either control aCSF or 100 nM FK960. 1/CV² was also determined for the same neurons from the amplitudes of 100 EPSPs recorded after 21-min exposure to either control aCSF or FK960. When changeover was made to control aCSF, there was a decrease of 6.5% in mean value for 1/CV² from 6.2 (range 3.2–9.0 in eight neurons, Fig. 4E) to 5.8 (range 2.3–9.2, Fig. 4E). The change in 1/CV² was not significant (P > 0.05, one-way ANOVA). However, in neurons exposed to 100 nM FK960, there was an increase of 53% in 1/CV² from 6.4 (range 2.8–9.6 in seven neurons, Fig. 4F) to 9.8 (range 5.4–16.3, Fig. 4F). The increase in 1/CV² after changeover to FK960 was significantly greater than seen in control aCSF (P = 0.007, one-way ANOVA). In control aCSF, mean quantum size was 0.33 ± 0.05 mV (n = 8) and was 0.37 ± 0.05 mV 21 min later. In a further seven neurons, mean quantum size was 0.39 ± 0.10 mV and was 0.42 ± 0.10 mV after 21-min exposure to 100 nM FK960. These changes in quantum size were not significant (P > 0.8, t test).

The frequency of spontaneous EPSPs, which includes spike-dependent and spike-independent responses, in four neurons was 0.65 spontaneous EPSP/s determined for the 10 min before changeover to 100 nM FK960 and 0.54 spontaneous EPSP/s for the period 20 to 30 min after exposure to FK960. The fall in spontaneous EPSP frequency was not significant (P = 0.25, paired t test).

Effect of FK960 on the GABAergic IPSC. To rule out the possibility that inhibition of GABA release mediates the action of FK960 on the EPSP, the effect of FK960 on the IPSC was examined. IPSCs were elicited in neurons voltage clamped at either −70 mV or −75 mV, after blockade of AMPA and NMDA receptors with 15 μM CNQX and 50 μM D-AP5, respectively, by stimulating the stratum radiatum. The IPSC consisted largely of a GABA A component (Fig. 5), although sometimes a smaller GABA B component was also present. In the experiment illustrated in Fig. 5, IPSC amplitude was 39 pA in control aCSF (Fig. 5A) and was essentially unchanged with an amplitude of 38 pA after 21 min in FK960 (Fig. 5B and C). Mean IPSC amplitude was 48 ± 10 pA (n = 3) in control aCSF and 46 ± 4 pA in FK960 (Fig. 5D); the difference in three experiments was not significant (P = 0.9, paired t test).

Effect of FK960 on EPSP. The increase in EPSP amplitude following exposure to FK960 was not accompanied by a significant change in τEPSP (Fig. 6C). In four experiments (in which the pipette solution contained 4 mM QX314 to prevent spiking), there was an increase in EPSP amplitude from 98.3 ± 14.5 pA to 158.3 ± 15 pA (P = 0.047, paired t test) after 20 to 22 min exposure to 100 nM FK960. There was an increase in τEPSP from 22.2 ± 3.1 ms to 28.4 ± 6.1 ms, which was not significant (P = 0.23, paired t test).

In experiments in which the slice was exposed to 2 mM BDP-12 (synthesized by Fujisawa Pharmaceutical Co. as FR212436) for 5 to 10 min, EPSP amplitude increased from 73.7 ± 8.9 pA (n = 3) to 239.7 ± 49.7 pA, and τEPSP increased significantly (P = 0.046, paired t test) from 21.5 ± 3.7 ms to 46.7 ± 5.4 ms (Fig. 6B). When exposed to 200 μM BDP-12 for 20 min, the increase in EPSP amplitude from 84 ± 36.6 pA (n = 3) to 181.3 ± 46.8 pA was similar in magnitude to that seen in FK960; τEPSP increased from 15.0 ± 2.2 ms to 22.4 ± 5.0 ms, although neither change reached statistical significance (P > 0.2, paired t test).
Effect of FK960 on EPSP Amplitude in the Presence of α7nAChR Antagonists. The α7nAChR receptor antagonists methyllycaconitine (10 and 100 nM) and α-bungarotoxin (300 nM) were studied for their action on the enhancement of the EPSP by FK960. Methyllycaconitine (100 nM) not only completely blocked the action of FK960 on the EPSP, but appeared to be without any action on its own (Figs. 7A and 8). In four CA1 neurons, mean EPSP amplitude remained unchanged at 2.9 ± 0.4 mV after 21 min in methyllycaconitine-aCSF (Figs. 7A and 8A). In another five CA1 neurons, mean EPSP amplitude was unchanged at 2.6 ± 0.3 mV after 21-min exposure to 100 nM FK960 in methyllycaconitine-aCSF (Figs. 7A and 8B). Clearly, none of these changes were significantly different (P > 0.05, one-way ANOVA). EPSP slope (P > 0.05, one-way ANOVA) and 1/CV² (a measure of the mean number of quanta released) was determined from 100 EPSPs recorded prior to solution changeover (precontrol) and compared with that determined from the same number of EPSPs recorded after 20-min exposure to either control aCSF (E) or FK960 (F). The neurons exposed to control aCSF showed a mean decrease of 6.5% in 1/CV² (E), whereas a mean increase of 53% was seen in the group-exposed FK960 (F).

Fig. 5. Effect of FK960 on IPSC. 100 nM FK960 had no significant effect on the IPSC in CA1 neurons. Hippocampal slices were exposed to an aCSF (control aCSF) containing CNQX (15 μM) and D-AP5 (50 μM) to block AMPA and NMDA receptors, respectively. Stimulation of the stratum radiatum at 0.1 Hz in the presence of these antagonists elicited a response consisting of GABA<sub>A</sub> and GABA<sub>B</sub> components. The averaged record of 15 IPSCs recorded from a CA1 neuron voltage clamped at −75 mV in control aCSF (broken line) is shown in A; the IPSC had a peak amplitude of 39 pA. After 21-min exposure to FK960, the peak amplitude was 38 pA (B, solid line). The records recorded in control and FK960 are shown superimposed in C. The IPSC reversed at −255 mV (not shown). D, histograms of mean IPSC amplitude before (control) and after exposure to FK960 (FK960), in three experiments; there was no significant difference (P = 0.9).

Fig. 6. Effect of FK960 and BDP-12 on EPSCs. EPSCs (average of 15 consecutive sweeps) recorded in CA1 neurons, voltage clamped at −70 mV, increased in amplitude after exposure to BDP-12 at 200 μM (from 42 pA to 95 pA; A) and 2 mM (from 87 pA to 247 pA; B). When the control currents were scaled (dotted line) to the same peak amplitude seen in the presence of BDP-12, there was a clear slowing in τ<sub>EPSC</sub> that was significant at the higher concentration. In contrast, FK960 increased EPSC amplitude, in this neuron from 103 pA to 175 pA (average of 15 consecutive sweeps), without any significant change in τ<sub>EPSC</sub> (C); the scaled control trace (dotted line) is superimposed on the trace seen in FK960.
results were obtained with 300 nM methyllycaconitine aCSF (open symbols, Fig. 7A). Similar changes were observed in the firing rate of CA1 pyramidal neurons, mean EPSP amplitude increased by 11% from 1.8 mV in the control solution to 2.0 mV in the presence of methyllycaconitine-aCSF, but rose by 10% from a mean of 0.2 mV in the presence of FK960. This suggests that FK960 has no ac-
instantaneous action on EPSP amplitude. At 15 min, the bathing solution was exchanged for one containing either FK960 (filled symbols) or methyllycaconitine-aCSF (open symbols). Data in Fig. 3 are the FK960/no antagonist controls for comparison with the data in A and B. (P > 0.05, one-way ANOVA) values were similarly unchanged (Fig. 8, C and F).

When 10 nM methyllycaconitine was used, EPSP amplitude fell by 5% from 2.2 ± 0.2 mV (n = 3) after 21 min in methyllycaconitine-aCSF, but rose by 10% from a mean of 3.1 ± 0.4 mV (n = 3) when FK960 was included. None of these changes were significant (P > 0.05, one-way ANOVA).

α-Bungarotoxin (100 nM) has been shown to block the action of nicotine on central neurons (McGehee et al., 1995; Gray et al., 1996). In the experiments reported here, 300 nM α-bungarotoxin was used to ensure rapid block during the 15-min pretreatment period. Mean EPSP amplitude fell from 1.9 ± 0.3 mV (n = 5) to 1.6 ± 0.3 mV after 21 min in α-bungarotoxin-aCSF (Figs. 7B and 9A). In another five CA1 neurons, mean EPSP amplitude increased by 11% from 1.8 ± 0.2 mV in α-bungarotoxin-aCSF to 2.0 ± 0.3 mV in FK960 (Figs. 7B and 9B). These changes in EPSP amplitude in the two groups of experiments were not significantly different (P > 0.05, one-way ANOVA). The changes in EPSP slope and 1/CV² following exposure to FK960 after incubation with α-bungarotoxin-aCSF were also not significant when compared with control (P > 0.05, one-way ANOVA; Fig. 9, C–F).

Discussion

This study demonstrates that the increase in EPSP amplitude and slope seen in hippocampal CA1 neurons following exposure to FK960 can be accounted for by an increase in transmitter release. FK960 had no significant effect either on the passive membrane properties of CA1 neurons or on τEPSC. The action of FK960 on τEPSC was examined and compared with that of the cognitive enhancer BDP-12 (Arai et al., 1996b; Arai and Lynch, 1998). We confirmed that BDP-12 at millimolar concentrations increased τEPSC, but were unable to demonstrate a similar effect of 100 nM FK960. Receptor properties such as affinity and the kinetics of desensitization and deactivation are important factors controlling the amplitude and time course of synaptic current at fast excitatory AMPAergic synapses (Vyklicky et al., 1991; Edmonds et al., 1995; Arai and Lynch, 1996). The AMPA/kine benzoyl-piperidine and benzoyl-pyrrolidine compounds (Arai et al., 1994, 1996a,b; Arai and Lynch 1998) and aniracetam (Isaacson and Nicoll, 1991; Tang et al., 1991) increase the amplitude and duration of the EPSC and field potential by reducing receptor desensitization and/or slowing deactivation (Arai and Lynch, 1998). The finding that FK960 had no effect on τEPSC or quantal size suggests that the properties of postsynaptic AMPAergic glutamate receptors on CA1 neurons are not altered and rule out a significant postsynaptic action for FK960. It is interesting to note in this context that the nootropic agent nefiracetam differs from aniracetam in that it appears to target presynaptic acetylcholine receptors to enhance glutamate release in the hippocampus (Nishizaki et al., 2000).

There was no significant change in the frequency of spontaneous EPSPs at times when FK960 significantly increased evoked EPSP amplitude, suggesting that FK960 has no action on baseline transmitter release from nerve terminals. To confirm this, it will be necessary to determine miniature EPSP frequency in the presence of the sodium channel blocker tetrodotoxin to block all spike-dependent transmitter.
A further observation relevant to the presynaptic action of FK960 was the block by methyllycaconitine (Macallan et al., 1988) and α-bungarotoxin indicating that activation of the α7nAChR is an obligatory link in the action of FK960. The α7nAChR (Couturier et al., 1990; McGehee et al., 1995; McGehee and Role, 1995; Gray et al., 1996) is widely distributed in the mammalian central nervous system (Seguela et al., 1993; MacDermott et al., 1999; Whiteaker et al., 1999) and is located pre- and postsynaptically in the hippocampus, as well as in a number of other brain areas (McGehee and Role, 1995; Gray et al., 1996; MacDermott et al., 1999). It is of particular interest because it is highly permeable to calcium ions (McGehee and Role, 1995; McGehee et al., 1995; MacDermott et al., 1999) having a Pca/PNa in the order of 20 (Seguela et al., 1993), and is, therefore, likely to play an important role in modulation of transmitter release by acetylcholine (McGehee et al., 1995; Alkondon et al., 1996; Gray et al., 1996; Wonnacott, 1997; Radcliffe and Dani, 1998; MacDermott et al., 1999). However, at present, the source of acetylcholine involved in such an action, whether from septohippocampal afferents or local interneurons, is unclear (Alkondon et al., 1998). It should be noted that the septal cholinergic innervation of the hippocampus should be complete or nearly complete in the rats used in this study that were 50 to 100 g in weight (21–34 days after birth; Milner et al., 1983).

It is well known that neurotransmitter gated ion channels opened by glutamate and GABA can be further regulated by a variety of modulators. It seems that α7nAChRs are also amenable to modulation via a site on the α7 subunit (Schrattenholz et al., 1996; Albuquerque et al., 1997; Maelicke and Albuquerque, 2000). The present experiments do not enable a conclusion to be drawn as to whether or not FK960 acts at this site, but suggest that FK960 may exert its action by modulating the strength of cholinergic transmission via such a mechanism. One approach to test this would be to see if the enhancement of EPSC amplitude and mEPSC frequency by nicotine in CA1 neurons is further increased by FK960 and whether this is blocked by the monoclonal antibody FK1 (Schrattenholz et al., 1996).
We also found, in agreement with others, that the dose-response relationship for FK960 is bell-shaped (Yamazaki et al., 1996; Matsuoka and Satoh, 1998; Matsuya et al., 2000). It is not possible to determine whether higher concentrations of FK960 act at a site distinct from that targeted by lower concentrations, but it is interesting to note that the bell-shaped dose-response relationship for FK960 is seen in experiments on isolated brain slices (Matsuoka and Satoh, 1998; Matsuya et al., 2000) and intact animals (Yamazaki et al., 1996; Matsuya et al., 2000). It is also worth noting that FK960 acts at a site on this receptor, also has a bell-shaped dose-response relationship (normalized acetylcholine current amplitude versus 1-methyl-galan-thamine concentration; Schrattenholz et al., 1996), as does the action of nefracetam on 7nAChRs expressed in Xenopus oocytes (Nishizaki et al., 2000). In contrast, the dose-response relationship for the cognitive enhancer BDP-12 (percent increase in peak current versus BDP-12 concentration) has a sigmoidal shape (Arai et al., 1996b).

One hypothesis put forward for the mechanism of action of FK960 is that it activates, directly or indirectly, somatostatinergic neurons in the hippocampus (Yamazaki et al., 1996; Matsuoka and Satoh, 1998). These experiments showed that pretreatment of animals with somatostatin, which reduced somatostatin levels in the brain (Yamazaki et al., 1996) significantly attenuated the action of FK960 both in vitro (LTP in brain slices, Matsuoka and Satoh, 1998) and in vivo (reversal of scopolamine-induced impairment using passive avoidance and Morris water maze techniques; Yamazaki et al., 1996). Lesion experiments were included in the study of Yamazaki et al. (1996), which led to the further suggestion that serotonergic and perhaps not surprisingly, cholinergic neurons are involved in the action of FK960, although the detailed nature of the relationship between these transmitter systems still remains to be worked out. The present study, in addition, implicates glutamatergic neurons in the growing collection of transmitter systems at which FK960 appears to be active.

The slow time course with which the action of FK960 on transmitter release develops is of considerable interest. We cannot rule out the possibility that FK960 may change the activity of the 7nAChR, not by acting at an extracellular site on the receptor, but by penetrating the nerve terminal to act intracellularly. Clearly, the slow time course could be related to the kinetics with which FK960 enters the nerve terminal to interact with an intracellular receptor. However, this is unlikely given the ease with which FK960 is orally absorbed and enters the brain. An alternative hypothesis would be that FK960 interacts with a specific receptor to cause a build-up of an intracellular messenger or interferes with a constitutively active enzyme cascade and indirectly causes a gradual change in the levels of a phosphorylated product that regulates transmitter release. Recently, a similar slow increase in quantal transmitter release at a glutamatergic synapse, following adrenergic stimulation, has been reported. The mechanism involves activation of a nitric oxide-independent guanylyl cyclase, resulting in an accumulation of intraterminal cyclic GMP and activation of protein kinase G (Yao, 1999).

In conclusion, the data presented show that FK960 increased the number of quanta released in response to nerve impulses, thereby increasing the amplitude and slope of the EPSP with no effect on quantum size. The importance of this positive modulation of AMPAergic transmission in relation to other transmitter systems with regard to enhanced cognitive performance is unknown, although we have demonstrated that activation of 7nAChR is required for this particular action of FK960. It is well established that up-regulation of AMPAergic transmission by, for example, AMPAkines is sufficient in some circumstances to promote significant improvement in cognitive function in man (Lynch et al., 1996). Therefore, it might be anticipated that glutamatergic transmission enhanced by FK960 will contribute, either directly or indirectly, to a therapeutic benefit in memory performance.

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Send reprint requests to: Dr. Joseph P. Hodgkiss, Fujisawa Institute of Neuroscience, Department of Neuroscience, University of Edinburgh, 1 George Square, Edinburgh EH8 9AZ, UK. E-mail: Joseph.Hodgkiss@ed.ac.uk