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5-HT_2 receptor-mediated reversal of the inhibition of hippocampal long-term potentiation by acute inescapable stress

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ABSTRACT

The serotonergic system is known to modulate and mediate many of the central nervous system effects of stress. Here we investigated the ability of serotonergic agents to reverse the inhibition of the induction of hippocampal long-term potentiation (LTP) caused by prior exposure to inescapable stress. Elevated platform stress prevented the induction of LTP in the CA1 area of anaesthetized rats. An agent that increases extracellular 5-HT concentration, fenfluramine (5 mg/kg, i.p.) enabled the induction of LTP in previously stressed animals. Consistent with a role for enhanced activation of 5-HT_2 receptors, the facilitatory effect of fenfluramine was prevented by the 5-HT_2 receptor antagonist cinanserin (30 mg/kg). Agents that directly activate 5-HT_2 receptors, including the 5-HT_{2B} receptor agonist BW 723C86 (30 mg/kg) and the 5-HT_{2C} receptor agonist MK-212 (3 mg/kg), mimicked the restorative effect of fenfluramine. Fenfluramine also opposed inhibition of LTP caused by the NMDA-receptor antagonist D-AP5 (100 nmol, i.c.v.) which suggests that the facilitatory action of serotonergic agents is not restricted to stress-mediated inhibition of LTP. These findings support an important role for activation of 5-HT_2 receptors by systemically applied agents to enable recovery from the inhibition of LTP by stress.

1. Introduction

Acute stress dramatically affects synaptic plasticity in the hippocampus, prior inescapable stress profoundly and persistently inhibiting the induction of long-term potentiation (LTP) by high frequency stimulation (Shors et al., 1989; Diamond et al., 1994; Xu et al., 1997, for reviews see Diamond et al., 2005; Joels and Krugers, 2007). LTP in the hippocampus provides a model of synaptic memory mechanisms and dysregulation of its induction or maintenance may be of importance in stress-related psychiatric disorders such as depression where normal hippocampal function is often impaired (Campbell and Macqueen, 2004).

Many current and potential therapies for stress-related psychiatric disorders either directly or indirectly modulate the serotonergic system and neuronal plasticity in the hippocampus (Agid et al., 2007; Paizanis et al., 2007). Furthermore, exposure to inescapable stress has been reported to increase 5-HT output in several brain areas including the hippocampus (Joseph and Kennett, 1983; Vahabzadeh and Fillenz, 1994).

The serotonergic innervation of the hippocampus is derived from the 5-HT neurons of the median and dorsal raphe (Conrad et al., 1974). Serotonin has seven distinct 5-HT receptor families often mediating opposing actions (Barnes and Sharp, 1999). Hippocampal LTP has been shown to be affected in different ways by modulation of 5-HT receptor activation. In non-stressed animals, agents increasing 5-HT availability such as the selective serotonin reuptake inhibitors (SSRIs), fluoxetine (Shakesby et al., 2002) and fluvoxamine (Kojima et al., 2003), inhibit the induction of LTP at CA1 synapses, an effect mimicked by 5-HT_{1A} receptor agonists (Corradetti et al., 1992). Consistent with a predominantly inhibitory effect of 5-HT on LTP in the CA1 area, antagonists at 5-HT_3 (Staubli and Xu, 1995) and 5-HT_{2A} (Wang and Arvanov, 1998) receptors have been reported to enhance LTP. However, a facilitatory effect of activation of 5-HT_4 receptors on the induction of LTP of cell firing (Matsumoto et al., 2002) and on the maintenance of synaptic LTP by prevention of its reversal (Kemp and Manahan-Vaughan, 2005) has been described.

In contrast, little is known regarding the possible role of 5-HT receptors in modulating LTP in stressed animals. Consistent with the predominantly inhibitory effect of 5-HT on LTP in non-stressed animals, agents that reduce endogenously released 5-HT reverse the inhibition of LTP in stressed animals (Shakesby et al., 2002). However, treatment with agents that increase brain extracellular

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5-HT concentration also have been reported to overcome the inhibition of LTP by stress (Rocher et al., 2004; Dupin et al., 2006; Vouimba et al., 2006). Furthermore increased hippocampal 5-HT release has been postulated to promote adaptation to the adverse effects of stressors on behaviour (Graeff et al., 1996). This study further explored if activation of 5-HT receptors using systemically injected agents can affect the mechanisms maintaining the disruptive effects of acute stress on the induction of LTP. We found evidence that activation of 5-HT2 receptors by systemic administration of agents that either raised endogenous 5-HT or directly stimulated 5-HT2 receptors reversed the inhibition of LTP caused by relatively brief elevated platform stress. However, the facilitatory effect was not specific to stress-inhibited LTP.

2. Methods

2.1. Animals

Male Wistar rats (in-bred strain, Bioresources Unit, Trinity College Dublin) weighing between 240 and 360 g were used for all experiments. Rats were group-housed with food (standard rodent chow) and water available ad libitum. The animals were housed under a 12 h light–dark cycle with the room temperature maintained between 19 and 22 °C. At the end of experiment the animal was killed by cervical dislocation. All experimental procedures received local ethical approval, were licenced by the Department of Health and Children, Ireland and conform to the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Surgery and electrophysiological recording

Prior to surgery animals were anaesthetised with urethane (ethyl carbamate; 2.1 g/kg, i.p.). A heating blanket (Harvard Apparatus Homeothermic Blanket Control Unit) was used to maintain a temperature of between 36 and 38 °C. Lignocaine (10 mg, 1% adrenaline) was injected s.c. over the area of the skull where electrodes were to be implanted.

Monopolar recording electrodes and bipolar stimulating electrodes were used in each experiment and implanted as described previously (Xu et al., 1997). The electrodes were made in the laboratory from two lengths of Teflon coated tungsten wire (625 μm tungsten diameter, 750 μm total external diameter) twisted together. The recording electrode was positioned 3.4 mm posterior to bregma and 2.5 mm lateral to midline. The stimulating electrode was positioned 4.2 mm posterior to bregma and 3.8 mm lateral to midline. A screw which acted as a reference electrode was positioned 8.0 mm anterior to bregma and lateral on the opposite hemisphere (left) to that used for electrode implantation. The earth screw which acted as ground electrode was positioned 7.0 mm posterior to bregma and 5 mm lateral to midline. A stereotaxic apparatus was used to place the electrodes in the CA1 area of the dorsal hippocampus. Electrophysiological criteria (Leung, 1979) were used to determine the optimal electrode placement. Field excitatory post-synaptic potentials (fEPSPs) were recorded from the stratum radiatum following stimulation of the Schaffer collateral–commissural pathway. Test fEPSPs were evoked by a single square wave pulse of current at low frequency (0.033 Hz, 0.1 ms duration). The test stimuli evoked responses of between 50 and 55% maximum fEPSP amplitude. The high frequency stimulation (HFS) protocol to induce LTP comprised 10 trains of 20 pulses, interpulse interval of 5 ms (200 Hz) and intertrain interval of 2 s. The intensity of stimulation was raised to give 75% of maximum fEPSP amplitude during the HFS.

The mild inescapable stress protocol consisted of placing the animal on a clear platform (25 cm × 25 cm) at a height of approximately 150 cm from ground level. The room was brightly lit. The animal was closely monitored for behavioural signs of stress, quantified in terms of duration showing behavioural freezing, piloerection and number of fecal boli and urinary deposits, while it remained on the platform for 30 min. This protocol has been shown previously to raise plasma corticosterone concentration and to strongly block LTP induction in the CA1 region of the hippocampus (Xu et al., 1997; Shakesby et al., 2002). The animal was anaesthetised immediately following stress exposure. The interval between stress exposure and the first delivery of HFS was approximately 165 min.

2.3. Compounds

All drugs were dissolved in distilled water. (±)Tianeptine was provided by Servier (Paris, France). (±)Fenfluramine was purchased from Sigma (Dublin, Ireland). MK-212 (6-chloro-2-(1-piperazinyl)pyrazine hydrochloride), BW 723C86 (α-methyl-5-(2-thienylmethoxy)-1*H*-indole-3-ethanamine hydrochloride), mCPP (1-(3-chlorophenyl)piperazine hydrochloride) and D-AP5 (D-(−)-2-amino-5-phosphonopentanoic acid) were purchased from Tocris (Bristol, UK).

The doses of agents were chosen based on pilot dose-ranging experiments and previous research: fenfluramine (Trulson and Jacobs, 1976); tianeptine (Shakesby et al., 2002); cinanserin (Clineschmidt et al., 1978); mCPP (Samanin et al., 1979); BW 723C86 (Kennett et al., 1998); MK-212, a 5-HT2C receptor preferential agonist (Clineschmidt et al., 1978); D-AP5 (Hayes et al., 2008). Apart from mCPP, none of the drugs had a discernible effect on baseline synaptic transmission over a recording period of approximately 1 h. We carried out separate experiments with mCPP to determine the duration of the small depression of transmission (see Fig. 3A) in order to ensure that it did not interfere with the measurement of LTP magnitude.

2.4. Data analysis

Data are expressed in epochs of 10 min and as mean ± S.E.M. LTP is expressed as a percentage of the mean fEPSP amplitude of the 30 min baseline prior to the first HFS (HFS1). Since two sets of HFS (HFS1 and HFS2) were applied at a 90 min interval in stressed rats LTP magnitude 1 h (50–60 min epoch) after either HFS1 or HFS2 was

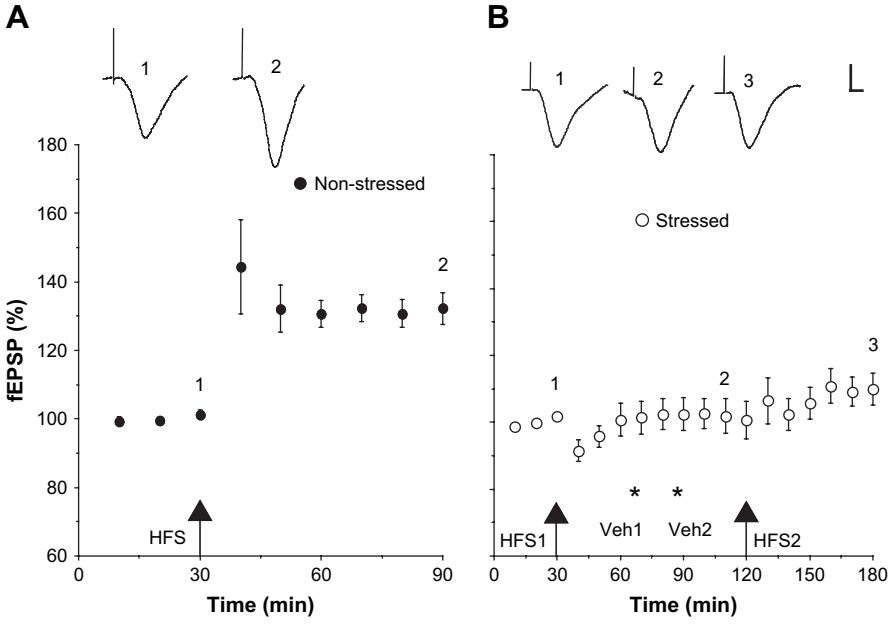
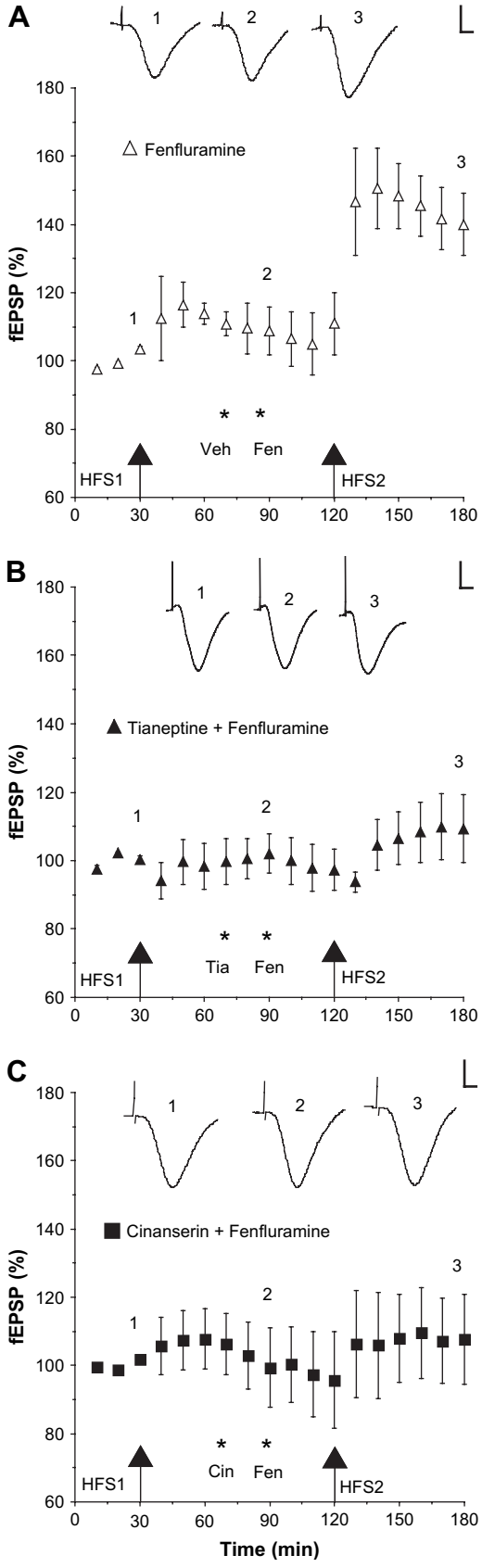


Fig. 1. Inhibition of LTP in the CA1 area of the anaesthetized rat by prior elevated platform stress. (A) Application of high frequency stimulation (HFS, arrow) in non-stressed control rats induced LTP of excitatory synaptic transmission ($p < 0.05$, $n = 8$). (B) Thirty-minutes exposure to an elevated platform stress prevented the induction of LTP by HFS. Two sets of HFS (HFS1 90 min before HFS2) failed to induce LTP ($p > 0.05$, $n = 5$). Animals received two vehicle (veh1 and veh2, i.p.) injections prior to HFS2 at the times indicated (*). Values are the mean ± s.e.m. Insets show typical traces of fEPSPs recorded at the times indicated. Vertical bar, 0.4 mV; horizontal bar, 5 ms.



compared with baseline (10 min preceding HFS1). For overall within group comparisons one-way ANOVA with repeated measures was carried out at the times indicated. For overall comparisons between groups a two-way ANOVA with repeated measures at the times corresponding to 1 h post-HFS1 and post-HFS2 was carried out. Detailed comparisons were made using paired or unpaired Student's *t*-tests, as appropriate, unless otherwise stated. A $p < 0.05$ was interpreted as significant.

3. Results

3.1. Elevated platform stress inhibits LTP

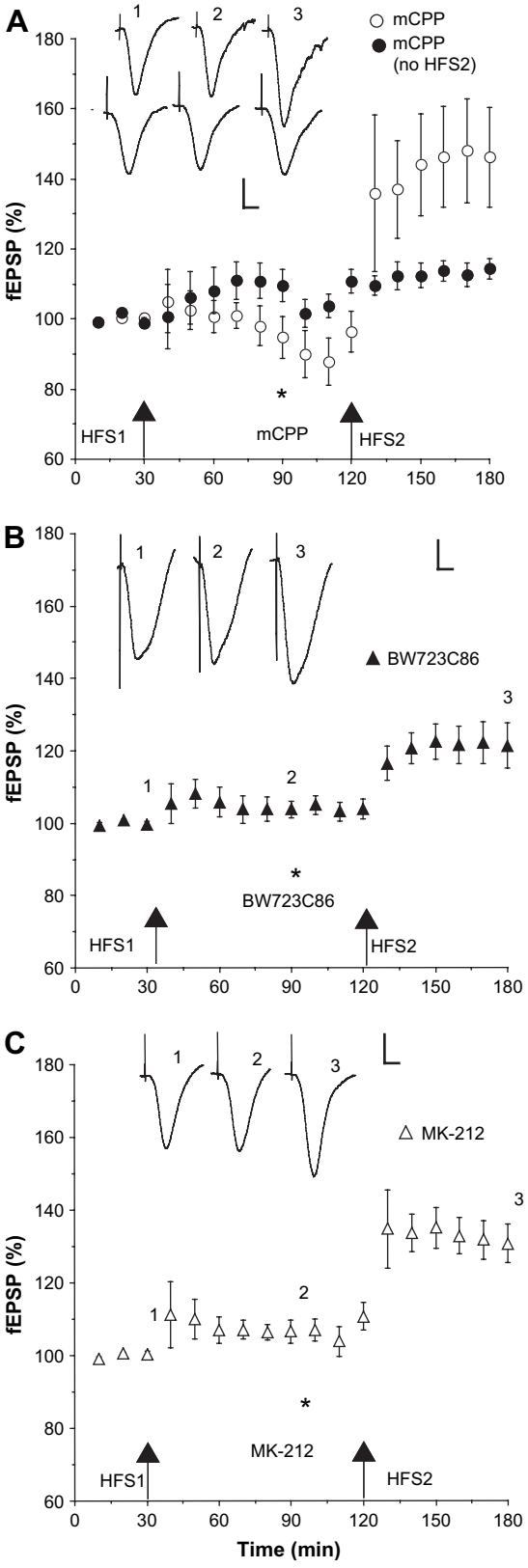
Pre-exposure to acute elevated platform stress inhibited the induction of hippocampal LTP under anaesthesia, confirming the studies of Xu et al. (1997) and Shakesby et al. (2002) (Fig. 1). Application of HFS in non-stressed animals in interleaved experiments induced a rapid onset and statistically significant stable increase in fEPSP amplitude (144.4 ± 13.7 and $132.3 \pm 4.6\%$ at 10 and 60 min post-HFS, respectively, $n = 8$, $F_{2,14} = 7.47$, $p < 0.05$) (Fig. 1A). Animals placed for 30 min on the platform showed behavioural signs of stress such as "behavioural freezing", piloerection, defecation and urination throughout this period. Thus, for example, whereas control, non-stressed animals did not show evidence of behavioural freezing or piloerection, stressed animals spent 27 ± 1 min of the 30 min in frozen posture with piloerection ($n = 5$). Immediately after the stress the animals were anaesthetised and electrodes implanted. Repeated HFS failed to induce LTP ($n = 5$, $F_{3,12} = 0.192$, $p > 0.05$). Thus, application of the first HFS (HFS1) did not induce a significant increase in synaptic transmission ($102 \pm 4.9\%$, $p < 0.05$ compared with 60 min post-HFS in non-stressed control animals). As this group acted as an interleaved control for the pharmacological challenges, animals then were given two vehicle injections, and at 90 min post-HFS1 a second HFS (HFS2) was applied. Again there was no significant increase in synaptic transmission ($109.8 \pm 4.7\%$, $p > 0.05$ compared with baseline) (Fig. 1B). These data clearly show that exposure to elevated platform stress immediately prior to anaesthesia persistently inhibited the induction of LTP for at least 4 h after the stress.

3.2. The 5-HT releasing agent fenfluramine overcomes stress-induced inhibition of LTP in a manner dependent on 5-HT$_2$ receptor activation

To determine if raising extracellular 5-HT concentration could overcome the inhibition of LTP induction by stress, fenfluramine, a potent 5-HT uptake inhibitor and releasing agent (Garattini et al., 1986; Mennini et al., 1991) was administered after the stress while the animal was under anaesthesia either alone or in combination with tianeptine, a 5-HT uptake enhancer (Fattaccini et al., 1990; Kamoun et al., 1989; Broqua et al., 1992; Kato and Weitsch, 1988) or the 5-HT$_2$ receptor antagonist cinanserin (Leysen et al., 1981; Fig. 2). Overall, there was a significant interaction between time and group ($F_{3,20} = 3.80$, $p < 0.05$) and a significant effect of time ($F_{1,20} = 17.72$, $p < 0.05$).

In the case of the fenfluramine alone group (Fig. 2A), the first HFS failed to induce LTP ($108.8 \pm 7.0\%$, $n = 7$, $p > 0.05$ compared

Fig. 2. An agent that raises extracellular 5-HT levels, fenfluramine, overcomes stress-induced LTP inhibition. (A) Administration of fenfluramine (Fen, 5 mg/kg, i.p.) 30 min before HFS2 overcame stress inhibition of LTP. Whereas platform stress prevented the induction of LTP by HFS1 ($p > 0.05$, $n = 7$) the second HFS-induced significant LTP ($p < 0.05$, $n = 7$). (B) Administration of the 5-HT uptake enhancer tianeptine (Tia, 2 mg/kg, i.p.) 10 min before fenfluramine (5 mg/kg, i.p.) prevented the recovery of LTP ($p > 0.05$, $n = 7$). (C) Stress prevented the induction of LTP by HFS ($p > 0.05$, $n = 5$). Pre-administration of the selective 5-HT2 receptor antagonist cinanserin (Cin, 30 mg/kg, i.p.) also prevented fenfluramine (5 mg/kg, i.p.) overcoming stress-induced LTP inhibition ($p > 0.05$, $n = 5$). Insets show typical fEPSPs recorded at the times indicated. Vertical bar, 0.4 mV; horizontal bar, 5 ms.



with baseline). At 60 min post-HFS1 fenfluramine was administered at a dose (5 mg/kg, i.p.). Thirty-minutes post-fenfluramine administration the second HFS-induced a statistically significant and stable LTP ($140.0 \pm 9.1\%$, $n = 7$, $p < 0.05$ compared with baseline or vehicle-treated control stressed animals) (Fig. 2A).

In order to help determine if the recovery of LTP post-fenfluramine administration in stressed animals was mediated via an increase in extracellular 5-HT, tianeptine was injected before fenfluramine (Fig. 2B). HFS1 failed to induce LTP ($102.0 \pm 5.8\%$, $n = 7$) in animals exposed to acute elevated platform stress. Tianeptine (2 mg/kg, i.p.) was administered 40 min post-HFS1 followed 20 min later by fenfluramine (5 mg/kg, i.p.). Application of HFS2 30 min after fenfluramine also failed to induce LTP ($109.3 \pm 9.9\%$). This is consistent with the proposal that an increase in extracellular 5-HT levels is the mechanism causing LTP recovery following treatment with fenfluramine.

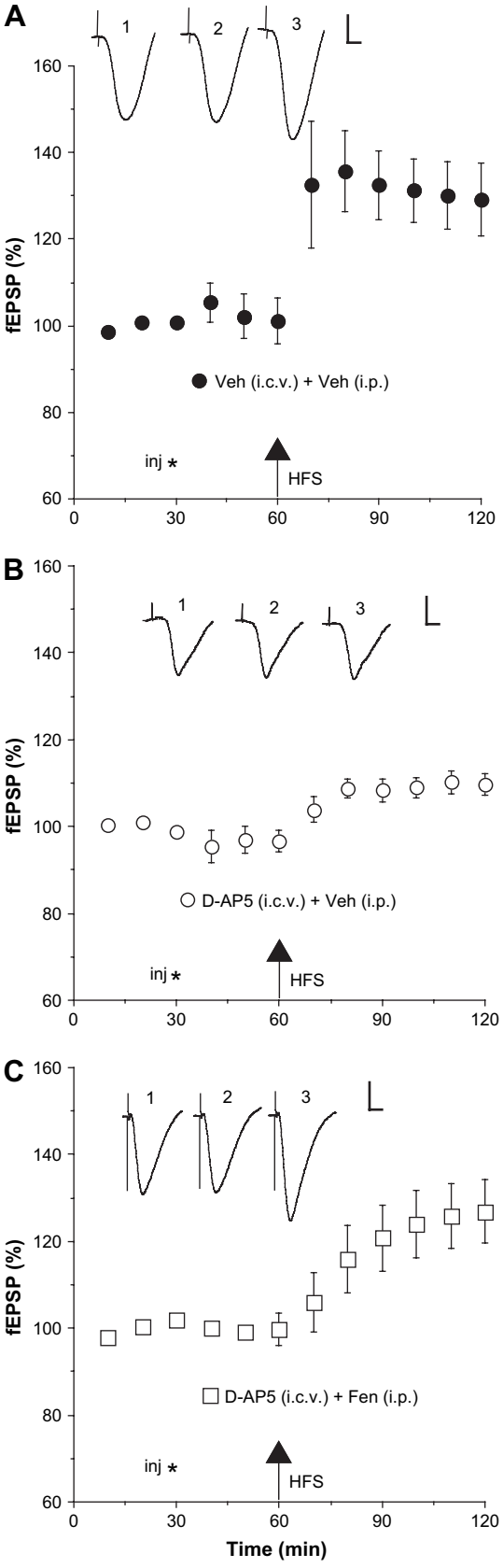
The possible role of 5-HT_2 receptor activation in the fenfluramine-induced recovery of LTP post-stress exposure was examined using cinanserin (Fig. 2C). Following HFS1 there was no significant increase in synaptic transmission ($99.4\% \pm 11.7\%$, $n = 5$) in animals exposed to acute elevated platform stress. At 40 and 60 min post-HFS1, cinanserin (30 mg/kg, i.p.) and fenfluramine (5 mg/kg, i.p.) were administered, respectively. Application of a second HFS 30 min later also failed to induce LTP ($107.7 \pm 13.1\%$).

3.3. 5-HT_2 receptor agonists overcome inhibition of LTP by stress

Since 5-HT_2 receptor activation appeared to be necessary for fenfluramine to promote recovery of LTP in stressed animals we investigated if 5-HT_2 receptor agonists could mimic the effect of fenfluramine. We examined the effect of mCPP, a 5-$HT_{2B/2C}$ receptor agonist (Porter et al., 1999; Whitton and Curzon, 1990), BW 723C86, a 5-HT_{2B} receptor preferential agonist (Kennett et al., 1996; Porter et al., 1999), and the 5-HT_{2C} receptor preferential agonist MK-212 (Conn and Sanders-Bush, 1987; Lee et al., 1992; Hemrick-Luecke and Fuller, 1996). In the case of mCPP we also examined its effect on baseline transmission. Overall, there was a significant interaction between time and group ($F_{4,22} = 3.24$, $p < 0.05$) and significant effect of time ($F_{1,22} = 19.65$, $p < 0.05$).

In rats exposed to acute elevated platform stress prior to mCPP injection, the application of HFS1 failed to induce LTP ($94.8 \pm 6.0\%$ at 60 min post-HFS1, $n = 6$, $p > 0.05$) (Fig. 3A). Administration of mCPP (10 mg/kg, i.p.) enabled LTP induction post-HFS2 ($146.2 \pm 14.3\%$, $p < 0.05$). As this dose of mCPP caused a brief reduction in the fEPSP amplitude we also examined the effect of mCPP on baseline transmission in rats exposed to acute elevated platform stress without applying the second HFS. In these animals application of HFS failed to induce LTP (109.5 ± 4.5, $n = 5$, $p > 0.05$). Subsequent administration of mCPP (10 mg/kg, i.p.) did not significantly alter synaptic transmission ($114.4 \pm 2.8\%$, at 30 min

Fig. 3. 5-HT_2 receptor agonists overcome stress-induced LTP inhibition. (A) The 5-HT_2 receptor agonist mCPP (10 mg/kg, i.p.) enabled the induction of LTP in previously stressed animals. Whereas the induction of LTP by HFS1 was inhibited in stressed animals ($p > 0.05$, $n = 6$), application of HFS2 30 min after mCPP induced significant LTP (open circles, $p < 0.05$). Administration of mCPP in stressed rats did not significantly affect baseline synaptic transmission (closed circles, $p > 0.05$, $n = 5$). Insets show typical fEPSPs recorded at the times indicated for mCPP with (upper traces) and without (lower traces) HFS2. (B) Another 5-HT_2 receptor agonist, BW 723C86 (30 mg/kg, i.p.), also enabled LTP induction in previously stressed rats. Stress prevented the induction of LTP by HFS1 ($p > 0.05$, $n = 5$) whereas HFS2 induced LTP 30 min after BW 723C86 injection ($p < 0.05$). (C) Similarly, administration of the 5-HT2 receptor agonist MK-212 (3 mg/kg, i.p.) overcame the inhibition of LTP by stress. Stress prevented the induction of LTP by HFS1 ($p > 0.05$, $n = 6$) but failed to inhibit LTP induction by HFS2 applied 30 min after MK-212 ($p < 0.05$). Insets show typical fEPSPs recorded at the times indicated. Vertical bar, 0.4 mV; horizontal bar, 5 ms.



post-injection, $n = 5$, $p > 0.05$ compared with 10 min pre-injection epoch) (Fig. 3A).

Administration of BW 723C86 also enabled LTP induction in previously stressed rats. Application of HFS1 failed to induce LTP ($103.8 \pm 2.3\%$ at 60 min post-HFS1, $n = 5$, $p > 0.05$) in rats exposed to the acute elevated platform. Application of the second HFS 30 min after injection of BW 723C86 (30 mg/kg, i.p.) induced LTP ($121.3 \pm 6.3\%$, $p < 0.05$) (Fig. 3B).

Similarly, MK-212 enabled LTP induction (Fig. 3C). In rats that were exposed to acute elevated platform stress the application of HFS1 failed to induce LTP ($106.5 \pm 3.2\%$ at 60 min post-HFS1, $n = 6$, $p > 0.05$). However, 30 min after injection of MK-212, HFS2 (3 mg/kg, i.p.) induced LTP ($130.8 \pm 5.3\%$ at 60 min post-HFS2, $p < 0.01$).

3.4. Effect of fenfluramine on LTP in non-stressed rats

Finally, the selectivity of fenfluramine for stress-inhibited LTP was tested next. Since fenfluramine did not enhance LTP induced by our standard HFS protocol in non-stressed animals (Ryan, Anwyl and Rowan, unpublished observations; Shakesby et al., 2002) and since this HFS-induced LTP is NMDA receptor-dependent (Doyle et al., 1996) we examined its effect on LTP that had been inhibited by the NMDA-receptor antagonist D-AP5 ($F_{2,24} = 2.52$ for the interaction between treatment and time, $p < 0.05$; $F_{2,24} = 26.91$ for the effect of time, $p < 0.05$) (Fig. 4). Thus in non-stressed animals injection of D-AP5 (100 nmol, i.c.v.) 30 min before the HFS significantly inhibited LTP ($110 \pm 2\%$, $n = 8$, $p < 0.05$ compared to vehicle-injected controls, $129 \pm 9\%$, $n = 6$). In contrast, in animals injected systemically (i.p.) with fenfluramine (5 mg/kg) at the same time as D-AP5 (100 nmol, i.c.v.) HFS now induced an LTP ($127 \pm 7\%$, $n = 5$, $p < 0.05$; $F_{2,27} = 0.06$ for the interaction between treatment and time, $p > 0.05$; $F_{2,27} = 16.53$ for the effect of time, $p < 0.05$) that was not significantly different from LTP in vehicle-injected controls at 60 min post-HFS ($p > 0.05$). Although the LTP was slow in onset in the fenfluramine and D-AP5 co-treated animals, the level of potentiation 10 min post-HFS was not significantly different from that evoked in either the group administered D-AP5 alone or the vehicle-injected controls ($p > 0.05$).

4. Discussion

The finding that fenfluramine reversed the inhibition of LTP induction by stress and that this reversal was prevented by the 5-HT_2 receptor antagonist cinanserin strongly indicates that increased activation of 5-HT_2 receptors promotes recovery from the inhibitory effect of acute stress. Moreover agonists at 5-HT_2 receptors also overcame the inhibition of LTP in previously stressed animals, providing further evidence that activity at this 5-HT receptor family can regulate the disruptive effects of stress on synaptic plasticity. The ability of fenfluramine to oppose LTP inhibition by an NMDA-receptor antagonist suggests that the facilitatory action of serotonergic agents is not restricted to stress-mediated inhibition of LTP.

Previously several groups have reported that prior exposure to acute inescapable stress is sufficient to block LTP induction in the CA1 area of the hippocampus in the awake/anaesthetized animals in vivo or ex vivo in brain slices (e.g. Shors et al., 1989; Diamond

Fig. 4. Fenfluramine overcomes NMDA-receptor antagonist inhibition of LTP in non-stressed rats. (A) In control, non-stressed animals injected (inj) with water i.c.v. (vehicle for D-AP5) and i.p. (vehicle for fenfluramine) the application of HFS 30 min later (arrow) induced significant LTP ($p < 0.05$, $n = 6$). (B) LTP was significantly attenuated in non-stressed animals that were injected with D-AP5 (100 nmol, i.c.v.) and vehicle i.p. ($p < 0.05$, $n = 8$). (C) Administration of fenfluramine (5 mg/kg, i.p.) at the time of injection of D-AP5 enabled the induction of LTP ($p < 0.05$, $n = 5$). Insets show typical fEPSPs recorded at the times indicated. Vertical bar, 0.4 mV; horizontal bar, 5 ms.

et al., 1994; Xu et al., 1997). The present findings confirm that placement on an elevated platform causes a persistent inhibition of LTP induction in the CA1 area if the rat is anaesthetised immediately after the stress (Xu et al., 1997; Shakesby et al., 2002). In contrast, if the animal is allowed to adapt to the stress there is a rapid recovery of the ability to induce LTP (Xu et al., 1997). Studying the delayed inhibitory effect of stress under anaesthesia allowed us to explore possible mechanisms of overcoming the persistent disruptive effects of stress on synaptic plasticity.

Fenfluramine, an agent that causes a marked increase in the extracellular concentration of 5-HT centrally (Sabol et al., 1992; Trulson and Jacobs, 1976; Carboni and Di Chiara, 1989; Zaczek et al., 1990), enabled the induction of LTP in previously stressed animals. This suggests that elevation of endogenous extracellular 5-HT levels can overcome stress-induced inhibition of LTP induction. Evidence consistent with this mechanism, rather than another effect of fenfluramine being responsible for the enablement of the induction of LTP in previously stressed animals, was obtained using tianeptine, a 5-HT uptake enhancer, thereby opposing the effect of fenfluramine on extracellular 5-HT concentration (Fattaccini et al., 1990; Kamoun et al., 1989; Broqua et al., 1992; Kato and Weitsch, 1988, but see also Malagié et al., 2000). Although these authors reported that a dose of 10 mg/kg enhanced 5-HT uptake, we (Shakesby et al., 2002) and others (Kole et al., 2002; Vouimba et al., 2006) found that treatment with 5–10 mg/kg caused an increase in baseline hippocampal excitability and consequently we chose to study the lower dose of 2 mg/kg. It is therefore possible that non-5-HT-related mechanisms may account for the actions of tianeptine. However, low dose tianeptine treatment reversed the inhibition of LTP by stress in an enantiomer selective manner and its facilitatory action was prevented by the 5-HT uptake inhibitor fluoxetine (Shakesby et al., 2002). If the recovery of LTP caused by fenfluramine is indeed mediated by an increase in extracellular 5-HT the restorative effect should be dependent on activation of 5-HT receptors. We therefore focused our attention on the 5-HT receptor superfamily and the 5-HT_2 receptor in particular. The ability of the selective 5-HT_2 receptor antagonist cinanserin to prevent fenfluramine enabling the induction of LTP in previously stressed animals suggests an important function for 5-HT_2 receptor activation.

Additional evidence of a role for 5-HT_2 receptor activation in overcoming stress-induced LTP inhibition was provided by the results of the experiments using agonists for 5-HT_2 receptors. Thus three different 5-HT_2 receptor agonists, mCPP, BW 723C86 and MK-212, all mimicked the restorative effect of fenfluramine. The hippocampus expresses post-synaptic 5-HT_{2A} (Vaidya et al., 1997), 5-HT_{2B} (Sanden et al., 2000) and 5-HT_{2C} (Clemett et al., 2000; Abramowski et al., 1995) receptors. It is difficult to assign a dominant role to a specific subtype based on this study. mCPP, the major metabolite of the antidepressant trazodone (Haria et al., 1994), in addition to being a direct agonist at 5-$HT_{2B/2C}$ receptors can also act as a 5-HT releasing agent (Baumann et al., 1993). Since BW 723C86 and MK-212 have been reported to exert 5-HT_{2B} and 5-HT_{2C} subtype selectivity (Kennett et al., 1996; Porter et al., 1999; Conn and Sanders-Bush, 1987; Lee et al., 1992; Hemrick-Luecke and Fuller, 1996), it is possible that activation of either 5-HT_{2B} or 5-HT_{2C} receptors is sufficient to overcome stress-induced inhibition of LTP induction. This shared action of agonists at 5-HT_{2B} and 5-HT_{2C} receptors may be due to the ability of both subtypes of the 5-HT_2 receptor family to harness the same phospholipase C (PLC) molecular machinery. Activating the PLC system, releasing intracellular Ca^{2+} which subsequently activates kinases such as Ca^{2+}/calmodulin-dependent kinase II (CaMKII) (Malinow et al., 1989) may thereby facilitate LTP induction in stressed animals.

Because this is an in vivo model with systemic injection of drugs we cannot rule out extra-hippocampal sites of serotonin release or 5-HT_2 receptor activation as possible mediators of hippocampal LTP recovery in previously stressed animals. For example, Kim et al. (2001) described how amygdalar lesions prevent CA1-LTP and spatial memory impairment caused by stress exposure. However, activation of 5-HT_{2C} receptors facilitates NMDA receptor-mediated transmission and LTP in the basolateral amygdala (Chen et al., 2003). Intriguingly, 5-HT_2 receptor activation increases acetylcholine release in several brain areas including the hippocampus (Hirano et al., 1995; Zhelyazkova-Savova et al., 1999; Nair and Gudelsky, 2004) and given the potential facilitatory effect of acetylcholine on LTP induction, it would be of interest to determine if the reversal of stress inhibition of LTP by 5-HT_2 receptor agonists was cholinoceptor-dependent.

Acute aversive stress exposure activates the serotonergic system (Graeff et al., 1996; Chaouloff, 1993; De Kloet, 2000; Joels, 2001) and can increase 5-HT release in the hippocampus (Joseph and Kennett, 1983; Vahabzadeh and Fillenz, 1994; Wilkinson et al., 1996; Matsuo et al., 1996; Ge et al., 1997; Kirby et al., 1997). The pattern and extent of 5-HT release depends on the nature of the stress. For example, Storey et al. (2006) reported evidence that 5-HT release was increased in the dorsal hippocampus after single placement on an elevated plus-maze but only after repeated exposure to an elevated platform. Consistent with a stress-evoked increase in 5-HT release and an inhibitory effect of endogenous 5-HT on LTP in the CA1 area of the hippocampus, agents that can lower extracellular 5-HT concentration in the brain prevented the inhibition of LTP by elevated platform or predator stress (Shakesby et al., 2002; Vouimba et al., 2006). However, the present findings clearly indicate that increasing 5-HT_2 receptor activation by pharmacological means can also overcome the disruptive effect of elevated platform exposure on LTP induction and is consistent with reports on the effects of agents that raise extracellular 5-HT concentration (Rocher et al., 2004; Dupin et al., 2006; Vouimba et al., 2006). Thus 5-HT appears to exert state-dependent opposing effects on the induction of LTP; when induction is strong LTP is inhibited, for example by activation of 5-HT_{1A} receptors (Corradetti et al., 1992; Shakesby et al., 2002; Kojima et al., 2003), whereas when induction is weak LTP is enhanced by 5-HT_2 receptor activation, as found in the present study. Importantly, the ability of fenfluramine to prevent the inhibition of LTP caused by the NMDA-receptor antagonist D-AP5 in non-stressed animals indicates that the facilitatory effect is not restricted to stress-inhibited LTP. Since the mechanism of the protective effect of fenfluramine against the inhibitory effect of D-AP5, a competitive NMDA-receptor antagonist, is unclear, it will be of interest to determine if fenfluramine and 5-HT_2 receptor agonists can prevent the inhibition of LTP by other means, including non-competitive NMDA-receptor antagonists. Future experiments with local injection of drugs into different brain regions at different times relative to HFS and stress also should help elucidate the mechanisms of these interactions.

The current findings indicate that activation of 5-HT_2 receptors, either by increasing endogenous 5-HT levels or by selective agonists, can promote recovery from the disruptive effect of inescapable stress on synaptic plasticity in the hippocampus. Clearly, it will be important to investigate the long-term effects of stress and drug exposure in order to evaluate any implications for putative antidepressant properties. It is hoped that further study of the mechanisms of the facilitatory effect observed in these studies will aid in the development of potentially novel ways to promote recovery from the deleterious effects of stress.

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