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The azapirone metabolite 1-(2-pyrimidinyl)piperazine depresses excitatory synaptic transmission in the hippocampus of the alert rat via 5-HT_{1A} receptors

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Abstract

The effects of acute and repeated treatment with 1-(2-pyrimidinyl)piperazine (1-PP), a metabolite of the 5-HT_{1A} receptor ligand azapirones, were investigated on hippocampal excitatory synaptic transmission. Recordings of the electrically evoked field population excitatory post-synaptic potentials (e.p.s.p.s) were carried out in the stratum radiatum of the CA1 region of the dorsal hippocampus of alert rats. Acute i.p. administration of 1-PP transiently reduced the e.p.s.p. amplitude in a dose-dependent (0.25–1 mg/kg) manner. This effect was blocked by the 5-HT_{1A} receptor antagonists spiroxatrine (1 mg/kg) and MDL 73005EF (8-[2-(2,3-dihydro-1,4-benzodioxin-2-yl methylaminoethyl]-8-azaspirol[4,5]decane-7,9-dione methyl sulphonate, 2 mg/kg). Intrahippocampal administration of 1-PP (5 μg) evoked a transient reduction of the e.p.s.p. amplitude which was similar to that obtained with 5-HT (10 μg). 1-PP (0.25 mg/kg per day) administered for 9 days produced a gradual reduction in the daily pre-injection baseline e.p.s.p. amplitude coupled with a decrease in the acute response to the drug. The chronic baseline reduction was transiently reversed by spiroxatrine and full recovery to pretreatment levels was observed 4 days after the last 1-PP dose. These findings indicate that the previously reported reduction in the e.p.s.p. produced by the azapirone group of 5-HT_{1A} receptor ligands may be mediated in part by their metabolite 1-PP through activation of 5-HT_{1A} receptors.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); 1-(2-Pyrimidinyl)piperazine; Hippocampus; Excitatory synaptic transmission; Electrophysiology; 5-HT_{1A} receptor; Azapirone

1. Introduction

The azapirone 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptor ligands comprise a major class of anxiolytic agents (for reviews: Eison and Eison, 1994; Deakin, 1993; Dourish, 1987). They include compounds such as buspirone, gepirone, tandospirone and ipsapirone, and generally function as presynaptic agonists and post-synaptic partial agonists at the 5-HT_{1A} receptor. The 5-HT_{1A} receptor is G protein linked to a K^+ ion channel which opens and produces membrane hyperpolarisation on activation (Andrade et al., 1986). Presynaptically, the azapirones act at somatodendritic autoreceptors on 5-HT cell bodies and dendrites in the raphe nuclei to reduce neuronal activity (VanderMaelen et al., 1986). Postsynaptically, they mimic/block the inhibitory effect of 5-HT in structures such as the hippocampus (Andrade and Nicoll, 1985).

The primary hepatic metabolite of the azapirones is 1-(2-pyrimidinyl)piperazine (1-PP), which is known to accumulate in the brain at higher concentrations than its parent compounds (Caccia et al., 1982, 1983, 1985, 1986). 1-PP has been shown to possess anxiolytic activity in some animal anxiety models such as anticonflict tests (Gammans et al., 1986; Gower and Tricklebank, 1988) and stress evoked ultrasonic vocalisations (De Vry et al., 1993; Cullen and Rowan, 1994). Consequently, it has been considered to be a potential active metabolite of this class of anti-anxiety agents. In contrast, 1-PP, unlike its parent molecules, is inactive in animal models of depression such as stress-induced behavioural despair and learned helplessness and indeed appears to oppose their putative antidepressant

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effects as determined in these tests (Martin, 1991, Przegalinski et al., 1990).

Whereas the azapirones have a relatively high selectivity for 5-HT_{1A} receptors 1-PP is known to have 10–100 times higher affinity for α_2-adrenoceptors than for 5-HT_{1A} receptors (Mennini et al., 1987). In a number of in vitro and in vivo tests 1-PP has been shown to behave as an antagonist at α_2-adrenoceptors (Giral et al., 1987; Bianchi and Garattini., 1988; Bianchi et al., 1988; review: Mennini et al., 1987). Furthermore, the anticonflict effect of azapirones has been attributed to α_2-adrenoceptor antagonism due to metabolism to 1-PP (Gower and Tricklebank, 1988). However, many more recently developed selective 5-HT_{1A} receptor ligands display anxiolytic activity in animal models but are not metabolised to 1-PP. Thus, considerable controversy exists as to the role of 1-PP in mediating the therapeutic effects of azapirones and in particular, which receptors are involved.

Although most animal studies have focused on the acute effects of azapirones it is only after repeated treatment that they exert a therapeutic effect (Deakin, 1993). In order to determine their mechanism of action at a clinically more relevant time frame, a series of electrophysiological experiments has compared the effects of acute versus repeated treatment with these compounds on hippocampal 5-HT_{1A} receptor mediated inhibition of excitatory synaptic transmission in alert rats (O'Connor et al., 1989; Manahan-Vaughan et al., 1994b). It was reported that sub-chronic administration of either gepirone or buspirone produced a more marked and prolonged inhibitory effect at relatively low doses. There is preliminary evidence that acute 1-PP also produces a reduction in excitatory synaptic transmission in the hippocampus which is equivalent to that evoked by its parent compounds (Manahan-Vaughan et al., 1990). The present study investigated the receptor mechanism of this effect and the effect of repeated as opposed to single injections in order compare its profile with that of its parent molecules. This is of particular interest given the recent clinical finding in a study of the effects of buspirone treatment in a group of patients with generalized anxiety disorder that a high degree of correlation was found for improvement in anxiety/depressive symptoms and plasma levels of 1-PP rather than of the parent compound after long-term therapy (Tollefson et al., 1991).

2. Materials and methods

2.1. Surgery, electrode implantation and recovery

Male Wistar rats which weighed between 180–200 g at the time of surgery were individually housed in a thermoregulated environment (19–23°C) using a 12 h light/dark cycle. The procedure adopted was similar to that described previously (Manahan-Vaughan et al., 1994b). Anaesthesia was induced with pentobarbitone sodium (40 mg/kg, i.p) and then maintained by means of inhaled halothane (1%) mixed with 100% O_2 at a rate of 1 l/min.

Three stainless steel screws (1.5 mm diameter) were inserted into the skull via a drill hole without piercing the dura. The ground screw electrode and was placed 8 mm posterior to bregma and 4 mm right of the midline. The reference screw electrode was placed 4 mm anterior to bregma and 4 mm right of the midline, over the frontal sinus. A third screw which served as an anchor was placed opposite the ground screw, 8 mm posterior to bregma and 3 mm left of the midline. The stimulating and recording electrodes were made of teflon coated tungsten wires (50 μm inner diameter, 75 μm outer diameter). The recording electrode was inserted 3 mm posterior to bregma and 2.8 mm lateral to the midline. The bipolar stimulating electrode was inserted 4 mm posterior to bregma and 3.8 mm lateral to the midline. Each electrode was lowered into the stratum radiatum of the CA1 region of the dorsal hippocampus using electrophysiological criteria. The entire assembly was sealed and fixed to the skull with dental cement.

Further verification that the electrodes were in the CA1 region of the stratum radiatum was obtained post mortem using light microscopy on formalin fixed tissue.

Before commencing the experiments the animals were allowed between 7–10 days to recover from surgery. During the recovery period they were rehearsed in the experimental procedure of light restraint in the recording hammock in order to familiarise them with the recording environment.

2.2. Field e.p.s.p. recordings from the alert rat

The animal was placed in a gently restraining hammock, from which it could easily escape, and a flexible cable was inserted into an electrode socket while recording took place. During these periods the animal was in a still, alert state. The population field excitatory post-synaptic potential (e.p.s.p.) was employed as a measure of excitatory synaptic transmission in the dorsal hippocampus. Stable e.p.s.p.s were evoked by stimulating at low frequency (0.025 Hz) with single square wave pulses of 0.1 ms duration. As it was found that there was a direct linear correlation between e.p.s.p. slope and amplitude, the amplitude was taken as the main indication of excitatory synaptic transmission. The e.p.s.p. amplitude was taken as the voltage difference between its point of onset to the peak of the potential. The baseline e.p.s.p. data were obtained by averaging the response over a 20 min period. The e.p.s.p.s used for the experiments were 60–70% of the maximum amplitude obtainable at baseline, and ranged

in amplitude from 2 to 3.5 mV in response to stimuli of between 3 and 5 mA. In control animals the input-output curves relating stimulus intensity versus e.p.s.p. amplitude were found to be stable over several weeks (up to 6 months).

2.3. Protocols for drug treatment

In the acute studies, all of the compounds and control vehicles were administered i.p. immediately following measurement of the e.p.s.p. baseline. The effect was monitored at 5 min intervals (averaging three sweeps per sample) until there was clear evidence of onset of recovery or, in the case of vehicle injected controls, a minimum of 50 min. The peak effect was taken as the maximum reduction observed during this period. 1-(2-Pyrimidinyl)piperazine (Bristol-Myers Squibb, Evansville), 8-hydroxy-2(di-*n*-propylamino)tetralin (8-OH-DPAT, Research Biochemicals Inc., Natick) and MDL 73005EF (8-[2-(2,3-dihydro-1,4-benzodioxin-2-yl methylaminoethyl]-8-azaspiro-[4,5]decane-7,9-dione methyl sulphonate, Marion Merrell Dow, Strasbourg) were injected in a water vehicle (1 ml/kg). Spiroxatrine (Janssen Pharmaceutica, Beerse) was suspended in a sonicated water solution of 10% Tween 80 (1 ml/kg). Antagonists were administered 30 min prior to drug injection. The effects of the antagonists alone were assessed over a minimum of 1 h after dosing. In the test for partial agonism 1-PP was administered 1 min before 8-OH-DPAT. Compounds for intrahippocampal injection were dissolved in a water vehicle and the dosage required administered in a volume of 0.5 μl. The injection was delivered gradually over a period of 1 min to prevent disturbance of the tissue. An e.p.s.p. baseline was obtained for 20 min prior to injection and the baseline following injection monitored until recovery.

Separate acute experiments were carried out in order to determine if 1-PP also altered the core temperature in order to rule out an indirect effect of the agent on the e.p.s.p. amplitude. Baseline pre-injection and 30 min post-injection rectal temperatures were measured while the animal was gently manually restrained.

In the chronic study, animals were injected at the same time each day for 9 days. A dose which acutely produced a relatively small effect was chosen for this experiment in order to allow comparison with our previous studies on buspirone and gepirone (O'Connor et al., 1990; Manahan-Vaughan et al., 1994a,b). The 24 h baseline e.p.s.p. amplitude readings were taken immediately prior to the daily injection and were expressed as a mean of the e.p.s.p. amplitude observed on day 0 (the day prior to starting daily injection) + day 14 (recovery) values. Acute readings (during the chronic studies) were obtained 10 min following daily injection and at 5 min intervals thereafter until recovery occurred (or the corresponding period of time had elapsed, usually 60–90 min).

On day 10, spiroxatrine was administered (24 h after the final dose). Further baseline recordings were taken 4 days after the last injection in order to monitor for recovery (washout).

2.4. Statistics

The data were expressed as the mean % (or % reduction from) pre-injection baseline e.p.s.p. amplitude ± standard error of the mean (S.E.M.). Statistical significance of the difference between means was estimated using two-tailed paired and unpaired Student's *t*-tests in the acute studies. In chronic studies analysis of variance (ANOVA) with repeated measures was carried out. Data for all post day 1 measurements were included for the 24 h baseline e.p.s.p. amplitude analysis. The acute response to injection measured during the chronic studies was compared with the pre-injection baseline readings on that day for both drug and vehicle conditions. The effect of acute antagonist challenge 24 h after the final chronic injection was expressed as a percentage of the baseline e.p.s.p. amplitude on that day and compared with pre-injection values using the Student's *t*-test. The probability levels interpreted as statistically significant were $P < 0.001$ (***), $P < 0.01$ (**), $P < 0.05$ (*).

3. Results

3.1. Effect of acute administration of 1-PP on e.p.s.p. amplitude

Acute i.p. injection of 1-PP produced a transient reduction in the amplitude of the e.p.s.p. (Fig. 1). This was dose-dependent in the range 0.25–1 mg/kg (n = 4–6; Fig. 1c). As seen in Fig. 1a,b, this effect was transient, reaching a peak at 20–25 min, which in the case of 1 mg/kg 1-PP caused a reduction of e.p.s.p. amplitude to 70 ± 4% of pre-injection baseline levels ($n = 6$; $P < 0.01$) versus water controls (98 ± 2%, n = 6) with full recovery by 60 min following injection.

There was no change in the core temperature of animals which received 1 mg/kg 1-PP, a dose which produced a near maximal inhibitory effect on the e.p.s.p., (+0.1 ± 0.4°C at 30 min post-injection, $n = 4$), compared with water injected controls (+0.1 ± 0.4°C at 30 min after 1 ml/kg, $n = 4$, $P > 0.05$).

Acute intrahippocampal (i.h.) injection of 1-PP in a dose of 5 μg in 0.5 μl produced a reduction of 31 ± 3% in e.p.s.p. amplitude (Fig. 2). This effect was transient, reaching a maximum at 5–10 min post-injection and with recovery at 15–20 min post-injection ($P < 0.01$ compared to water injected controls, $n = 4$). By com-

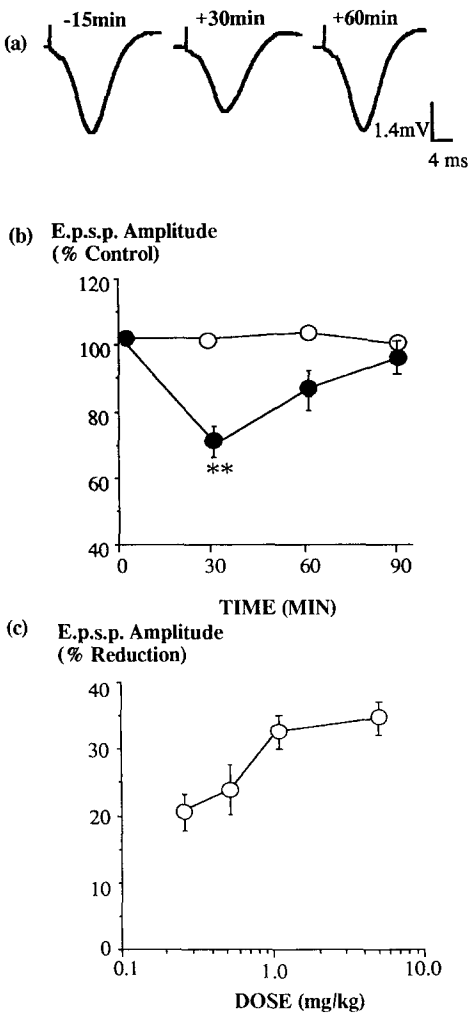

Fig. 1. Acute effect of i.p. injection of 1-PP on hippocampal excitatory transmission in the alert rat. (a) Effect of 1-PP (1 mg/kg, i.p.) on the e.p.s.p. recorded in the stratum radiatum of the CA1 region (original traces at $t = -15$, $+30$, and $+60$ min relative to time of injection). (b) Time-course for the acute effect of 1-PP (1 mg/kg, i.p., $n = 6$, black symbols; $^{**}P < 0.01$ versus water controls, $n = 6$, clear symbols) on the e.p.s.p.. Values are the mean percentages of pre-injection baseline control ± S.E.M. (c) Dose-response relationship for the inhibitory effect of systemic injection of 1-PP (0.25–5 mg/kg, i.p., $n = 4$–6 per point) on the amplitude of the electrically evoked e.p.s.p. Two doses were tested in some rats but an interval of at least 4 days was given between injections. Values are mean % pre-injection reduction ± S.E.M.

parison, acute intrahippocampal (i.h.) injected of 5-HT (10 μg in 0.5 μl water) produced a reduction of 46 ± 5% in e.p.s.p. amplitude (Fig. 2), which effect was transient, reaching a maximum at approximately 5 min post-injection with recovery by 15 min ($P < 0.001$ compared to water injected controls, $n = 4$).

3.2. Effect of 1-PP on the response to 8-OH-DPAT

Pretreatment with 1-PP (1 mg/kg, i.p.) before the injection of 8-OH-DPAT (50 μg/kg) produced a reduction of pre-injection baseline e.p.s.p. amplitude to 73 ± 2%. This effect was significantly less than the reduction of e.p.s.p. amplitude obtained with water + 8-OH-DPAT (to 57 ± 5%, $P < 0.05$, $n = 4$, Fig. 3a). The former reduction was similar to that found when 1-PP was given alone at this dose (Fig. 1b).

3.3. Effect of spiroxatrine on the response to 1-PP

When spiroxatrine was administered 30 min prior to 1-PP (1 mg/kg) there was no change in the e.p.s.p. baseline amplitude (104 ± 4% control, $n = 6$) whereas 1-PP following pretreatment with Tween 80, the vehicle for spiroxatrine, produced a reduction to 78 ± 6% of pre-injection e.p.s.p. baseline amplitude ($n = 6$, $P < 0.01$, Fig. 3b). When administered alone, this dose of spiroxatrine had no effect on e.p.s.p. amplitude (98 ± 2% pre-injection baseline e.p.s.p. amplitude, $n = 4$).

3.4. Effect of MDL 73005EF on the response to 1-PP

The effect of 1-PP (1 mg/kg, $n = 6$) was reduced from 72 ± 1% of pre-injection e.p.s.p. baseline amplitude to 91 ± 2%, when pretreatment with 2 mg/kg MDL 73005EF was used ($n = 4$, $P < 0.01$ compared to water pre-injected, Fig. 3b). When administered on its own, this dose of MDL 73005EF had no significant effect on e.p.s.p. amplitude (95 ± 2% pre-injection baseline e.p.s.p. amplitude, $n = 4$).

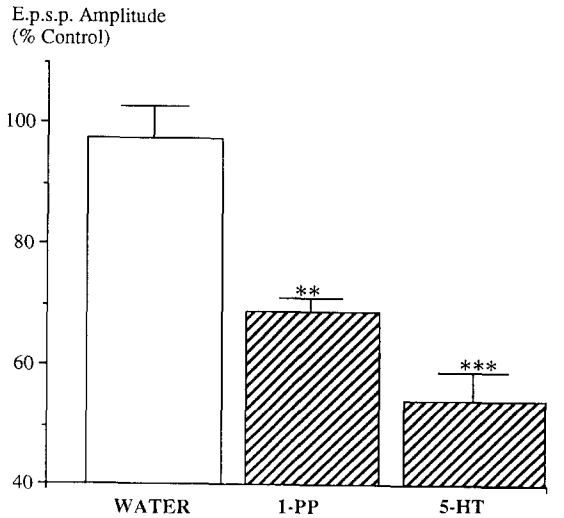

Fig. 2. Acute effect of intrahippocampal injection of 1-PP or 5-HT on excitatory synaptic transmission in the CA1 region of the alert rat. The bar chart illustrates the peak effect of 1-PP (5 μg in 0.5 μl water, i.h., $n = 4$, $P < 0.01$ versus water controls), 5-HT (10 μg in 0.5 μl water, i.h., $n = 4$, $P < 0.01$ versus water controls) or an equivalent volume of water on the amplitude of the e.p.s.p. Values are the mean percentages of pre-injection baseline control values ± S.E.M.

3.5. Effect of repeated treatment with 1-PP (0.25 mg / kg)

3.5.1. Chronic 24 h baseline e.p.s.p. amplitude

There was an overall significant difference in the amplitude of the e.p.s.p. between treatment groups ($F(1,11) = 23.9$, $P < 0.001$, Fig. 4) and treatment days ($F(1,5) = 39.8$, $P < 0.001$; from day 1 to day 10). The interaction between group and day was also significant indicating a differential effect of 1-PP depending on day of repeated treatment ($F(1,2) = 10.1$, $P < 0.001$). A steady decline in 24 h chronic baseline amplitude was thus noted throughout the study, which had reached a minimum by day 10. On day 7 a reduction to 83.6 ± 9% of chronic baseline values occurred ($n = 6$, $P < 0.05$ compared to water treated controls). This decreased further to 59.6 ± 10% ($P < 0.001$; $n = 6$) on day 10. On day 14, full recovery of baseline was noted (100 ± 5%, $P > 0.01$ versus water controls, $n = 6$).

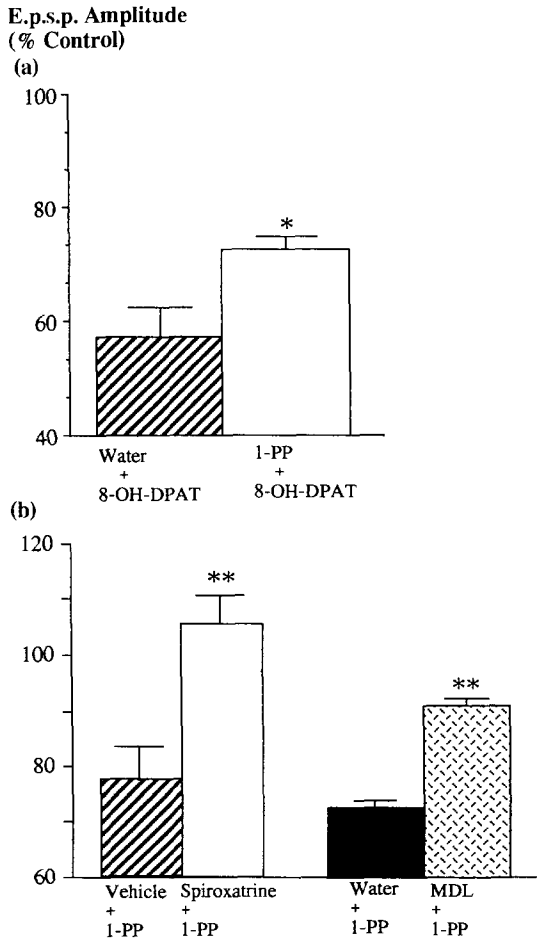

Fig. 3. Receptor pharmacology of the acute effect of 1-PP on hippocampal excitatory synaptic transmission in the alert rat. (a) The effect of 8-OH-DPAT (50 μg/kg, i.p.) on the amplitude of the e.p.s.p. after pretreatment with water (hatched bar, $n = 4$) or 1-PP (1 mg/kg, i.p., $n = 4$, clear bar; $^{*}P < 0.05$). (b) The effect of 1-PP (1 mg/kg, i.p.) on the amplitude of the e.p.s.p. after pre-treatment with spiroxatrine (1 mg/kg, clear bar, i.p., $n = 6$; $^{**}P < 0.01$ compared to treatment with vehicle + 1-PP, $n = 4$, hatched bar) or MDL 73005EF (2 mg/kg, stippled bar, $n = 4$; $^{**}P < 0.01$ compared to treatment with water + 1-PP, $n = 4$, black bar). Values are the mean percentages of pre-injection baseline control values ± S.E.M.

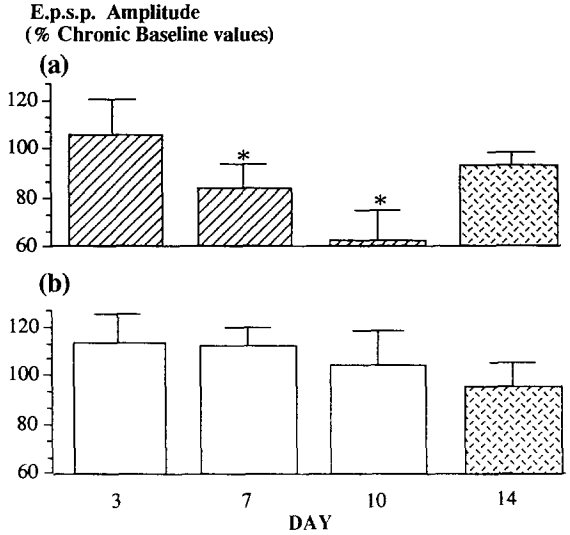

Fig. 4. The effect of chronic treatment with 1-PP (0.25 mg/kg, i.p.) on hippocampal excitatory synaptic transmission in the alert rat. 1-PP (a, $n = 6$; hatched bars) and water (b, $n = 9$; clear bars) were given daily on days 1–9. On day 14 (stippled bar) no injection had been given. Values were measured 24 h after the previous injection and are expressed as the mean ± S.E.M. percentage of the chronic 24 h baseline e.p.s.p. amplitude. $^{*}P < 0.05$ compared to water injected controls.

3.5.2. Acute response to 1-PP in chronically treated animals

A decrease in the acute response to the daily dose of 1-PP was observed at the time when there was a

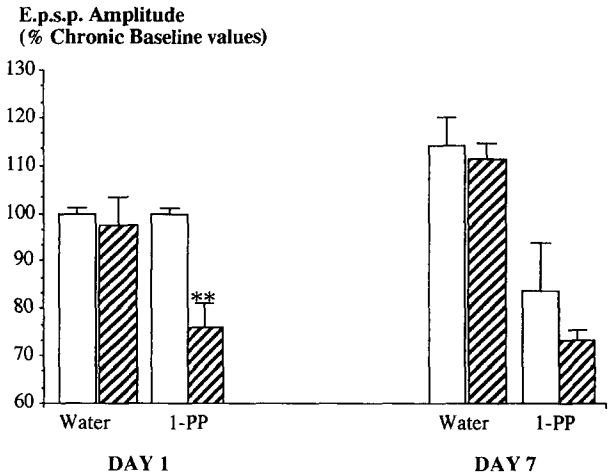

Fig. 5. Comparison of the acute effect of 1-PP (0.25 mg/kg) or water on hippocampal e.p.s.p. amplitude on days 1 and 7 of repeated daily treatment with 1-PP (0.25 mg/kg, i.p., $n = 6$) or an equivalent volume of water ($n = 6$; clear bars; pre-injection values, hatched bars: post-injection values). Values are the means ± S.E.M. percentage of the chronic 24 h baseline e.p.s.p. amplitude. $^{**}P < 0.01$ compared to control (pre-injection) 24 h baseline.

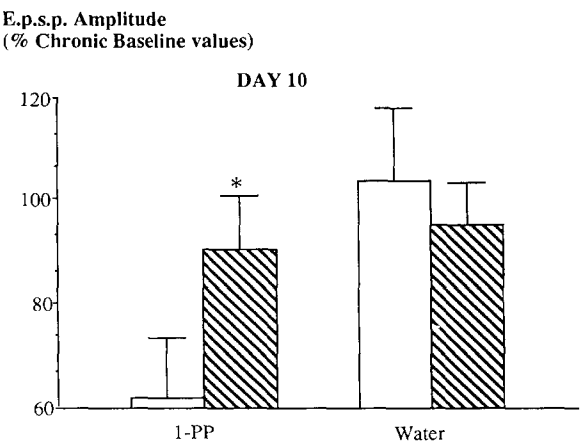

Fig. 6. Effect of acute injection of spiroxatrine (1 mg/kg, i.p.) in 1-PP (0.25 mg/kg) or water injected animals ($n = 6$, clear bars: pre-spiroxatrine, hatched: post-spiroxatrine values). Thirty min following spiroxatrine administration on day 10 of the chronic study, the 24 h baseline was restored to control levels in the 1-PP-treated animals. Values are the means ± S.E.M. percentage of the chronic 24 h baseline e.p.s.p. amplitude. $^{**}P < 0.01$ versus pre-injection control values.

reduction in the 24 h e.p.s.p. amplitude (Fig. 5). It was found that on day 1, the reduction of e.p.s.p. amplitude was 24 ± 4% ($P < 0.01$; $n = 6$) of pre-injection levels whereas by day 7 the acute response was 11 ± 3% of the 24 h baseline which was not significant ($P > 0.05$, $n = 5$).

On day 22 (13 days after the last chronic injection) of the study, administration of 1 mg/kg 1-PP caused a decrease in the e.p.s.p. amplitude of 36 ± 3% which is similar to that seen after a single injection at this dose level (Fig. 1c).

3.5.3. Effect of spiroxatrine on the chronic 24 h baseline e.p.s.p. amplitude

On day 10 of the study the e.p.s.p. baseline amplitude monitored following an acute injection of spiroxatrine (1 mg/kg, i.p., $n = 6$). 30 min post-injection, spiroxatrine had completely restored the 24 h baseline values ($P < 0.05$, compared to pre-injection levels Fig. 6). This reversal was transient, and full return to pre-injection baseline values had occurred by 60 min post-injection. Injection of this dose in the water control animals had no effect.

4. Discussion

The findings of this study indicate that repeated administration of the azapirone metabolite 1-PP produces a gradual and prolonged reduction in basal excitatory amino acid-mediated synaptic transmission in the hippocampus, which is accompanied by the development of tolerance to the acute inhibitory effect of 1-PP. These data are very similar in profile, pharmacology and time-course to that previously found in comparable studies with 5-HT_{1A} receptor ligands such as buspirone, gepirone and 8-OH-DPAT. Systemic acute injection of 1-PP in this study resulted in a transient dose-dependent reduction of the e.p.s.p. evoked in the dorsal hippocampal CA1 region of alert rats which was similar to that seen following application of 5-HT_{1A} receptor agonists (O'Connor et al., 1989, 1990; Manahan-Vaughan et al., 1994a,b). Consistent with this, intrahippocampal injection of 1-PP mimicked the effects obtained with intrahippocampal injection of 5-HT and of selective 5-HT_{1A} receptor agonists (O'Connor et al., 1990). Furthermore, the effect of 1-PP could be blocked by application of the 5-HT_{1A} receptor antagonists spiroxatrine (Nelson and Taylor, 1986) and MDL 73005EF (Hibert et al., 1988; Van den Hooff and Galvan, 1991). Overall, the results offer strong evidence that 1-PP may be an active metabolite of the azapirones and that its mechanism of action in the hippocampus occurs via 5-HT_{1A} receptors.

Although the present study did not directly assess whether or not 1-PP mediates the effect of its parent compounds the data are consistent with it playing a significant role. When injected acutely i.p., 1-PP was 3–4 times more potent in reducing the amplitude of the hippocampal e.p.s.p. than gepirone or buspirone were in previous comparable studies, but produced a similar maximum effect (O'Connor et al., 1990; Manahan-Vaughan et al., 1994b). Similar to gepirone (Manahan-Vaughan et al., 1994b), 1-PP partly blocked the inhibitory effect of 8-OH-DPAT, indicating possible partial agonist activity at 5-HT_{1A} receptors in vivo. This contrasts with a previous report that iontophoretic administration of 1-PP to CA3 pyramidal neurones at a dose level which reduced baseline firing rate had no effect on the response to presumed 5-HT_{1A} receptor activation (Blier et al., 1991). Given the much lower affinity of 1-PP for 5-HT_{1A} receptors compared to its parent molecules (6–100 times lower, Mennini et al., 1987) our findings may seem surprising. However, in rats the peak brain concentrations of 1-PP achieved 30–60 min after oral and intravenous dosing with gepirone or buspirone are 5–10 times higher than in plasma and exceed concentrations of the azapirones by a factor of about 2 (Caccia et al., 1982, 1983, 1985, 1986). Indeed ex vivo experiments indicate that 1 h after acute buspirone (10 mg/kg, p.o.) there is enough 1-PP present to at least partly account for the level of occupancy of 5-HT_1 receptors observed (Gobbi et al., 1991). However, since direct intrahippocampal injection of either buspirone or gepirone produces a reduction in e.p.s.p. amplitude (O'Connor et al., 1990) not all hippocampal depressant effects of these agents are due to 1-PP.

It is possible that 1-PP might act indirectly to pro-

duce the 5-HT_{1A} receptor-dependent reduction in e.p.s.p. amplitude. 1-PP has a relatively high affinity for α_2-adrenoceptors in vitro and an ability to block α_2-adrenoceptor-mediated effects in vivo at doses in the range used in the present study (see Introduction). Of particular relevance is the ability of 1-PP to block inhibitory α_2-adrenoceptors located on serotonergic nerve terminals in rat cortex (Gobbi et al., 1990). Block of α_2-adrenoceptors has been reported to increase hippocampal 5-HT release in freely moving rats (De Boer et al., 1994) and 1-PP has been shown to increase hippocampal 5-HIAA levels in vivo (Grazia De Simoni et al., 1990). Indeed the block of a behavioural effect of 8-OH-DPAT by 1-PP has been proposed to involve antagonism of α_2-adrenoceptors (Dursun and Handley, 1993). In contrast to these biochemical and behavioural studies, previous electrophysiological research in alert rats have been unable to see any significant effect of the α_2-adrenoceptor antagonist idazoxan (1–3 mg/kg, i.p.) on basal e.p.s.p. amplitude in CA1 (O'Connor et al., 1990 and unpublished observations) or dentate gyrus (Sara and Bergis., 1991). The lack of effect of α_2-adrenoceptor block may be due to the low density of adrenoceptors in the stratum radiatum of the CA1 region (Young and Kuhar, 1980) or a low basal noradrenergic tone to those sites regulating 5-HT release in this region under the present recording conditions. Thus, the 1-PP-induced 5-HT_{1A} receptor-mediated reduction in excitatory synaptic transmission seems unlikely to involve block of α_2-adrenoceptors.

Repeated administration of 1-PP over 9 days produced a gradual decrease in the baseline e.p.s.p. amplitude to 60% control levels, which was accompanied by an apparent loss of the acute response to 1-PP ($\sim$ 20% reduction at this dose in drug naive animals). The chronic baseline reduction in e.p.s.p. amplitude was transiently reversed 24 h after the last injection by spiroxatrine indicating that it was due to a basal activation of 5-HT_{1A} receptors at a time when the levels of 1-PP would be expected to be very low (Caccia et al., 1986) although the possible accumulation of 1-PP in the brain cannot be excluded. The loss of the acute response to 1-PP is likely to be due to occlusion (i.e. a baseline effect) rather than to tolerance. The profile of effects obtained with repeated application of 1-PP closely corresponds to that observed following administration of the azapirones gepirone and buspirone and the selective 5-HT_{1A} receptor agonist 8-OH-DPAT (O'Connor et al., 1989, 1990; Manahan-Vaughan et al., 1994a,b). Although the exact site of action for these effects is not known, it is probable that they share common mechanisms (see Manahan-Vaughan et al., 1994a,b, for discussion).

In conclusion, the findings of this study suggest that 1-PP is capable of acting as an active metabolite of the azapirones in the hippocampus after both acute and repeated administration and that its effects on excitatory amino acid-mediated transmission are mediated via 5-HT_{1A} receptors. The present results lend support to the view that 1-PP may be an important mediator of the therapeutic effects of the azapirones (Tollefson et al., 1991).

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