ELSEVIER

European Journal of Pharmacology 323 (1997) 59–68

ejp

Effect of repeated ipsapirone treatment on hippocampal excitatory synaptic transmission in the freely behaving rat: role of 5-HT_{1A} receptors and relationship to anxiolytic effect

Lin Xu [a], Roger Anwyl [b], Jean De Vry [c], Michael J. Rowan [a,*]

[a] *Department of Pharmacology and Therapeutics, Zoology Building, University of Dublin, Trinity College, Dublin 2, Ireland*
[b] *Department of Physiology, University of Dublin, Trinity College, Dublin 2, Ireland*
[c] *Institute of Neurobiology, Troponwerke GmbH & Co. KG, Berliner Strasse 156, D-51063 Cologne, Germany*

Received 10 October 1996; accepted 31 December 1996

Abstract

The effects of acute and repeated treatment with the 5-HT_{1A} receptor ligand ipsapirone on hippocampal excitatory synaptic transmission and in an ultrasonic vocalization anxiety test were investigated in the rat. Synaptic responses in the CA1 region of the dorsal hippocampus of alert, freely behaving male Wistar rats were reduced after acute injection of ipsapirone (1 or 2 mg/kg, i.p.). This effect was prevented by pretreatment with the 5-HT_{1A} receptor antagonist WAY-100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclo-
hexanecarboxamide trihydrochloride, 0.25 or 0.5 mg/kg, i.p.) but not by the 5-HT-depleting agent *para*-chlorophenylalanine (300 mg/kg per day for 3 days, i.p.). WAY-100635 (0.1–0.3 mg/kg, i.p.) also blocked the acute anti-aversive effects of ipsapirone (3 mg/kg, i.p.) in the anxiety test. Repeated administration of ipsapirone (1 or 2 mg/kg per day for 7–8 days, i.p.) produced a gradual reduction in baseline synaptic transmission which was transiently reversed by WAY-100635 (0.25 mg/kg, i.p.). Ipsapirone (1 mg/kg per day for 7 days) produced a gradual and sustained reduction in the duration of vocalizations in the anxiety test which paralleled the reduction in baseline synaptic responses in the same animals. The data indicate that with repeated administration of ipsapirone, a prolongation and enhancement of the 5-HT_{1A} receptor-mediated reduction in hippocampal excitatory synaptic transmission occurs. This delayed effect may contribute to the sustained anxiolytic and/or antidepressant effect of ipsapirone.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); Ipsapirone; WAY-100635; *p*-Chlorophenylalanine; Hippocampus; Excitatory synaptic transmission; 5-HT_{1A} receptor; Excitatory postsynaptic potential; Ultrasonic vocalization; Anxiolytic

1. Introduction

The pyrimidinylpiperazine anxiolytics such as buspirone, gepirone, ipsapirone and tandospirone have marked acute inhibitory effects on neuronal transmission in the rat hippocampus. In vitro they share the ability to hyperpolarize rat hippocampal pyramidal cells by the activation of 5-HT_{1A} receptor G-protein-linked K^+ channels (Colino and Halliwell, 1987; Andrade and Nicoll, 1987). 5-HT_{1A} receptors may also be located on glutamatergic terminals, thereby contributing to the depressant effects on excitatory transmission in the hippocampus (Schmitz et al., 1995). The decrease in spontaneous firing rate of hippocampal pyramidal cells in anaesthetised animals (Blier and De Montigny, 1987; Sprouse and Aghajanian, 1988) and the reduction in rhythmic slow wave (theta) activity (Hirose et al., 1990; Coop and McNaughton, 1991) and excitatory synaptic transmission (O'Connor et al., 1990; Manahan-Vaughan et al., 1994a,b) in this region seen in awake rats, have been suggested as a possible mechanism for their anxiolytic or antidepressant effects.

Since these drugs must be taken repeatedly before their therapeutic efficacy becomes apparent (Harto et al., 1988; Glitz and Pohl, 1991) it is necessary to examine their subchronic effects when investigating their possible mechanism of action. Following repeated application of these 5-HT_{1A} receptor ligands an adaptation of the 5-HT system may be produced (Bohmaker et al., 1993; De Vry, 1995). For example, there is evidence that the net output of the inhibitory serotonergic drive to the hippocampus may be

* Corresponding author. Tel.: (353-1) 702-1567; Fax: (353-1) 671-3507; e-mail: mrowan@mail.tcd.ie

0014-2999/97/$17.00
PII S0014-2999(97)00022-8

increased as a result of reduced negative feedback in the dorsal raphe nucleus (Blier and De Montigny, 1987; Godbout et al., 1991). One method of assessing the functional significance of any such increase is to compare the acute and subchronic effects of these compounds on hippocampal excitatory synaptic transmission in vivo. In the case of buspirone and gepirone there is evidence for an enhancement and a prolongation of its acute depressant effects on the amplitude of hippocampal field excitatory postsynaptic potential (e.p.s.p.) after repeated treatment in awake, lightly restrained animals (O'Connor et al., 1989; Manahan-Vaughan et al., 1994b). This is consistent with the view that adaptive changes take place. In contrast, there does not appear to be a change in the effect of buspirone on hippocampal theta activity during long-term treatment in freely behaving rats (Zhu and McNaughton, 1991).

The main aim of the present study was to determine if acute and repeated systemic treatment with ipsapirone affected hippocampal excitatory synaptic transmission in freely behaving rats in a manner similar to that of buspirone and gepirone in lightly restrained animals and if its effects were sensitive to the novel, highly selective silent 5-HT_{1A} receptor antagonist WAY-100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride, Forster et al., 1995). In order to determine the relationship between its effect on synaptic transmission and its anxiolytic effects, reversibility of the anti-aversive effects of acute ipsapirone by pretreatment with WAY-100635 was investigated and the time course of the anti-aversive effects of repeated ipsapirone treatment was compared with its effects on the e.p.s.p. The test chosen, ultrasonic vocalizations following a mildly aversive foot shock, is known to be sensitive to anxiolytic agents including ipsapirone (De Vry et al., 1993), but a direct comparison of the anti-aversive and electrophysiological effects produced with a subchronic treatment schedule in the same animals has not been investigated previously.

2. Materials and methods

2.1. Animals

Male Wistar rats, weighing 200–250 g at the beginning of the subchronic study and 250–300 g for acute study, were used. After implantation of the electrodes, animals were housed individually with free access to water and food in an established animal house having a 12 h light/dark cycle and thermoregulated environment. The experiments did not begin until the animals had fully recovered from anaesthesia and surgery and were well used to being handled (usually about 2 weeks). The animal care and experimental protocol was licensed by the Department of Health, Ireland and approved by the local German authorities (acute ultrasonic vocalization test).

2.2. Surgery and electrode implantation

The method of electrode implantation used here was similar to that described previously in detail (O'Connor et al., 1989, 1990). The surgery was carried out under pentobarbitone sodium (60 mg/kg, i.p.) anaesthesia. When necessary, the rat was given another injection of pentobarbitone sodium (20 mg/kg, i.p.), about 40–60 min after the first injection.

Three stainless-steel screws (1.5 mm diameter) were inserted into the skull through a drill hole without piercing the dura. One served as a ground electrode (7 mm posterior to bregma and 5 mm left of the midline), another acted as an anchor (opposite the ground screw, 7 mm posterior to bregma and 5 mm right of the midline) and the third served as the reference electrode (8 mm anterior to bregma and 1 mm left of the midline).

Recording and stimulating electrodes were made by gluing together a pair of twisted Teflon-coated tungsten wires (50 μm inner diameter, 75 μm outer diameter). The recording electrode was inserted 3.4 mm posterior to bregma and 2.5 mm right of the midline and 2.5 mm below the surface of the dura and the stimulating electrode was inserted 4.2 mm posterior to bregma and 3.8 mm right of the midline and 2.8 mm below the dura, through separate drill holes. The correct placement of the electrodes in the stratum radiatum of the CA1 region of the dorsal hippocampus was verified by electrophysiological criteria (Leung, 1979) and by post-mortem examination. The electrode socket assembly was fixed onto the skull with dental cement.

2.3. E.p.s.p. recordings in freely behaving animals

At the same time each day after the implantation of the electrodes, the rats were put in a recording box ($32 \times 21 \times 20$ cm, made of transparent red Perspex) for 1 h. During this period the socket assembly was connected to a preamplifier via a flexible wire. Usually, on the first of these training sessions, the rat remained in a 'frozen' state (no movement but alert) for the first 10 min before exploring the box for almost 30 min. After 1 week of daily training, there was no evidence of behavioural 'freezing' and the time of active exploration was reduced to the first 5–10 min. The rats became very tame and well used to being handled during the training period.

Excitatory synaptic transmission in the dorsal hippocampus was measured by recording the population field e.p.s.p. which was evoked by stimulating the Schaffer collaterals/commissural fibres in the stratum radiatum of the CA1 region. The stimulus was a square-wave constant-current pulse of 0.1–0.2 ms duration (rate 0.033 Hz). The field e.p.s.p. amplitude, defined as the size of the initial negative peak from baseline, had stabilised by about 10 days after implantation. At this stage the input-output curve (relation between stimulus intensity and field e.p.s.p. amplitude) was recorded in order to determine the maxi-

mum field e.p.s.p. amplitude. During the experiments the stimulus intensity was set at a level which evoked a field e.p.s.p. amplitude of 60–70% of the maximum. The control baseline field e.p.s.p. amplitude was obtained by calculating the mean amplitude over 20 min (usually 40 sweeps). The effects of drugs on e.p.s.p. amplitude were measured as a percentage of this value. For the subchronic studies the control baseline field e.p.s.p. amplitude on the day when subchronic treatment was commenced (day 1) was used as the reference level.

In order to minimise possible effects of changes in motor activity and arousal on the field e.p.s.p. amplitude the behaviour of the animals in the recording box was monitored continuously. The rats were left undisturbed for at least 15 min before recording commenced. Recordings were made when the animals were normally in a still but alert state and unless otherwise stated there was no obvious change in the animals' behaviour during this period. After the initial period of adaptation to the recording box there was no significant change in brain temperature (data not shown). Recording took place at approximately the same time each day so as to minimise possible effects of diurnal variation. All of the electrophysiological studies were carried out in Dublin.

2.4. Ultrasonic vocalization

The effect of acute administration of ipsapirone on foot shock-induced ultrasonic vocalization and reversibility of the anti-aversive effects of ipsapirone by pretreatment with WAY-100635 were investigated in animals following the method described by De Vry et al. (1993). In short, rats received a series of 20 inescapable foot shocks (2 mA, scrambled shocks of 2 s) through an electrical grid according to the following schedule: a series of five shocks, each spaced 8 s apart, was repeated 4 times, in standard operant chambers during five consecutive daily training sessions. This schedule was the minimum needed to evoke a reliable ultrasonic response. A test session started with a series of five shocks, as described above, and immediately thereafter the total duration of ultrasonic (17–29 kHz) vocalization was measured for 5 min, two additional shocks were given after 2 and 4 min, respectively. Rats reaching the baseline responding requirements (>150 s of ultrasonic vocalization on two consecutive test sessions) were randomly assigned to one of four treatment groups ($n = 9$–10 per group; vehicle/vehicle, vehicle/ipsapirone, WAY-100635/vehicle or WAY-100635/ipsapirone). Rats were initially tested with 0.1 mg/kg WAY-100635 (or vehicle), and 1 week later, following a second randomization, tested again with 0.3 mg/kg WAY-100635 (or vehicle). Pretreatment with WAY-100635 (or vehicle) was given 15 min before treatment with ipsapirone (or vehicle). Testing occurred 15 min after the latter application. These acute studies were carried out in Cologne and the doses used were slightly different from those used in the other studies reported here.

The effect of subchronic administration of ipsapirone (see below for schedule) on the response to aversive stimulation was also evaluated in a set of animals which had chronic indwelling electrodes. This allowed us to assess drug-induced change in the affective state, as measured by ultrasonic vocalization to foot shock, in parallel with the electrophysiological responses. Each day, approximately 24 h after the previous foot shock, baseline measures of the e.p.s.p. amplitude were taken over a 20 min period in the recording box. Immediately after this the animal was placed in the dark compartment ($25 \times 25 \times 25$ cm) of a light-dark box and foot shock was applied via stainless-steel bars which formed the floor of the box (for a more detailed description see Cullen and Rowan, 1994). The foot shock was generated by a constant-current (2 mA) unscrambled stimulus generator for 5 s. Following the shock ultrasonic vocalizations at 22 kHz were recorded over a period of 3 min. The percentage time spent vocalizing was calculated as an indicant of the aversive response. The high-frequency calls were picked up with an acoustic transducer (Polaroid Instruments electrostatic transducer, range 20–200 kHz) placed in the centre of the roof of the dark compartment. The input from the transducer was passed through a pre-amplifier which in turn was connected to a band-pass filter which rejected any signal outside the frequency range of interest (20–25 kHz). The subchronic studies were carried out in Dublin.

2.5. Drug treatment

In the present studies all of the compounds and control vehicle injections were given i.p. The acute effect of each compound was monitored at 5-min intervals (mean of three sweeps) until the field e.p.s.p. had recovered to pre-injection levels. In the vehicle-injected controls the field e.p.s.p. was monitored for at least 1 h. The peak effect was taken as the maximum change of the field e.p.s.p. amplitude.

The following drugs were used in this experiment: ipsapirone, WAY-100635 (synthesized by the Chemistry Department, Bayer, Wuppertal, Germany) and *para*-chlorophenylalanine (PCPA, Sigma). All compounds were dissolved in double-distilled water.

In the acute studies, WAY-100635 was given 15 min before the injection of ipsapirone or water. PCPA was administered at the same time each day for 3 days. On the 4th day, 24 h after the last injection the effect of ipsapirone was examined.

In the subchronic studies ipsapirone was given as a single daily injection for 7 days at 1 mg/kg and for 8 days at 2 mg/kg. The water-injected controls were treated for 7 days. The rats were given 0.25 mg/kg WAY-100635 i.p. on day 8, 24 h after the last daily injection of either 1 mg/kg ipsapirone or water. Body weight gain was monitored throughout these subchronic studies. No significant differences were seen between the groups (data not shown).

2.6. Statistics

Values are expressed as the mean percentage pre-injection baseline field e.p.s.p. amplitude ± S.E.M. or, in the subchronic studies, mean percentage of the field e.p.s.p. amplitude of day 1 values ± S.E.M. Statistical significance of the difference between means was estimated using two-tailed paired and unpaired *t*-tests in the acute studies. Two-way analysis of variance with repeated measures was used in the subchronic studies, followed by paired and unpaired *t*-tests for individual group comparisons. Acute effects of ipsapirone and WAY-100635 on shock-induced ultrasonic vocalization were analyzed by means of analysis of variance, followed by a Tukey pair-wise comparison test. The mean duration of ultrasonic vocalization was correlated with mean amplitude of the e.p.s.p. using the Pearson product-moment correlation coefficient, r.

3. Results

3.1. Acute effect of ipsapirone on the field e.p.s.p. amplitude in the freely behaving rat

Ipsapirone had an acute depressant effect on the field e.p.s.p. amplitude in the stratum radiatum CA1 of the dorsal hippocampus in freely behaving rats. The field e.p.s.p. amplitude decline was transient, the peak occurring at about 15 min and recovering to pre-injection baseline by 20–25 min post-injection (Fig. 1B and Fig. 2A; decreasing to 91.5 ± 2% pre-injection baseline, 15 min after a 1 mg/kg ipsapirone injection, $P < 0.05$, $n = 6$ and 86.6 ± 3.2% 15 min after injection with 2 mg/kg ipsapirone, $P < 0.05$, $n = 4$, compared with 100.1 ± 2.3%, 15 min after injection of 1 ml/kg water vehicle, $n = 4$, Fig. 1A).

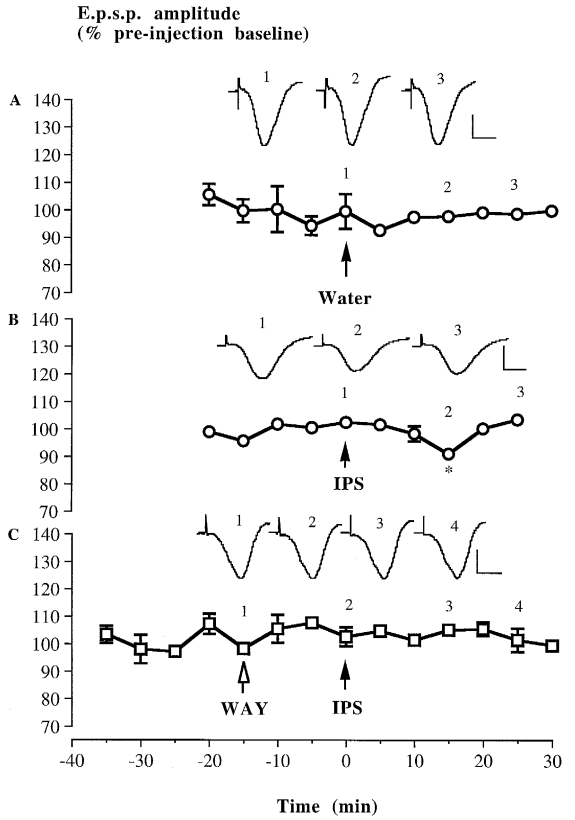

Fig. 1. Effect of acute ipsapirone treatment on hippocampal excitatory synaptic transmission in freely behaving rats. (A) Water (1 ml/kg, i.p., $n = 6$) injected at time 0 (arrow) had no significant effect on the amplitude of the e.p.s.p. (B) Ipsapirone injection (arrow, 1 mg/kg, i.p., $n = 6$) produced a transient 9% reduction in the e.p.s.p. amplitude. (C) Pretreatment for 15 min with the 5-HT_{1A} receptor antagonist WAY-100635 (0.25 or 0.5 mg/kg, i.p., $n = 4$, combination of two at each dose) prevented the depressant effect of 1 mg/kg ipsapirone (closed arrow). Values are the mean ± S.E.M. * $P < 0.05$ compared to water. Insets show typical traces of the field e.p.s.p. at the times indicated by the numbers. Vertical calibration bar: 1 mV; horizontal calibration bar: 5 ms.

3.2. Effect of WAY-100635 pretreatment on the electrophysiological response to acute ipsapirone treatment in the freely behaving rat

There was no change in the field e.p.s.p. amplitude over a 45 min period following the injection of 1 mg/kg ipsapirone in rats which had been treated with 0.25 or 0.5 mg/kg WAY-100635 15 min previously (Fig. 1C, $n = 4$, combining two at each dose, $P < 0.01$, compared to ipsapirone alone; $P > 0.05$, compared to pre-injection baseline). These doses of WAY-100635 had no effect on baseline transmission when followed for up to 1 h after injection ($n = 4$, see also Fig. 5A).

3.3. Effect of WAY-100635 pretreatment on the anti-aversive effect of acute ipsapirone treatment in the ultrasonic vocalization anxiety test

Acute effects of WAY-100635 and ipsapirone on shock-induced ultrasonic vocalization are shown in Table 1. The first experiment, combining 0.1 mg/kg WAY-100635 with ipsapirone, revealed a main pretreatment effect of WAY-100635 ($F(1,35) = 5.03$, $P < 0.05$), a main treatment effect of ipsapirone ($F(1,35) = 52.65$, $P < 0.001$), and an interaction effect between pretreatment and treatment ($F(1,35) = 4.89$, $P < 0.05$). Ipsapirone (3 mg/kg) reduced mean ultrasonic vocalization by 86% as compared with vehicle/vehicle control. Pretreatment with WAY-100635 attenuated significantly the anti-aversive effects of ipsapirone and was devoid of any activity by itself, suggesting that the antagonism by WAY-100635 was behaviourally specific. A similar picture was obtained in the case of pretreatment with WAY-100635 at the higher dose of 0.3 mg/kg (pretreatment effect: $F(1,35) = 6.76$, $P < 0.02$; treatment effect: $F(1,35) = 30.88$, $P < 0.001$; interaction effect: $F(1,35) = 5.11$, $P < 0.05$). Again, ipsapirone (3 mg/kg) was found to profoundly reduce ultrasonic vocalization (88% reduction as compared with vehicle/vehicle control) and pretreatment with WAY-100635

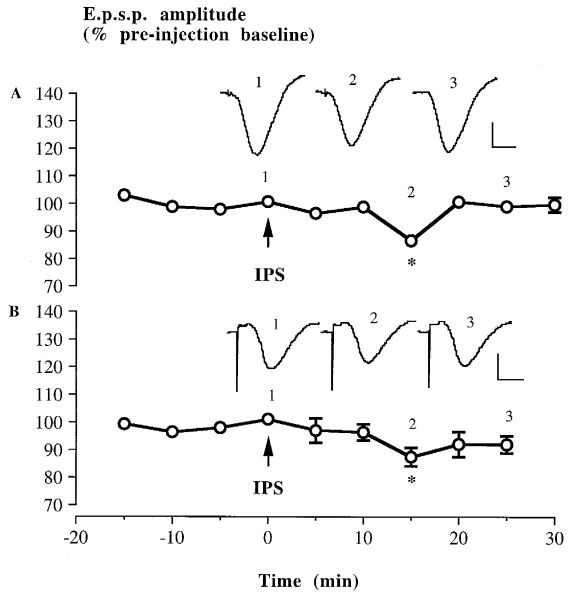

Fig. 2. Lack of antagonism of the acute depressant effect of ipsapirone by the 5-HT-depleting agent PCPA. (A) Acute injection of ipsapirone (arrow, 2 mg/kg, i.p., $n = 4$) produced a 13% reduction in the e.p.s.p. amplitude. (B) Pretreatment for 3 days with PCPA (300 mg/kg, i.p., $n = 4$) did not affect the reduction (12%) in e.p.s.p. amplitude produced by ipsapirone (arrow, 2 mg/kg, i.p.). Values are the mean ± S.E.M. * $P < 0.05$ compared to water. Insets show typical traces of the field e.p.s.p. at the times indicated by the numbers. Vertical calibration bar: 1 mV; horizontal calibration bar: 5 ms.

greatly reduced the anti-aversive effects of ipsapirone in a behaviourally specific manner (33% reduction as compared with vehicle/vehicle control).

3.4. Effect of PCPA pretreatment on the acute effect of 2 mg/kg ipsapirone

In PCPA-pre-treated animals (300 mg/kg per day for 3 days, a dose regimen which has been reported to deplete hippocampal 5-HT levels by the fourth day, Schreiber et al., 1994), acute injection of 2 mg/kg ipsapirone still markedly reduced the field e.p.s.p. amplitude with a similar time course to non-pre-treated controls, peaking at about 15 min (Fig. 2B; $n = 4$, 88.4 ± 3.9% 15 min after injection, $P > 0.05$ compared to animals injected with 2 mg/kg ipsapirone alone, Fig. 1C). PCPA treatment had no effect on baseline transmission. For example, 24 h after the third PCPA injection the e.p.s.p. amplitude was 98.2 ± 7.8% of the pre-drug period ($n = 4$, $P > 0.05$, compared to 100.5 ± 3.8% in water-injected animals, $n = 4$).

3.5. Effect of subchronic administration of ipsapirone on the field e.p.s.p. amplitude in the freely behaving rat

Daily treatment with 1 or 2 mg/kg ipsapirone resulted in a gradual, dose-dependent and long-lasting decrease in basal excitatory synaptic transmission in the hippocampus.

Treatment with 1 mg/kg per day ipsapirone for 7 days ($n = 10$) gradually produced a reduction in the 24 h baseline e.p.s.p. amplitude (Fig. 3B, $F(1,12) = 7.68$, $P < 0.05$, compared to water-injected controls, Fig. 3A). Thus, 24 h after the second day's injection the mean of the field

Table 1
Effects of pretreatment with WAY-100635 on the acute anti-aversive properties of ipsapirone in the ultrasonic vocalization anxiety model

Pretreatment (mg/kg, i.p.)	Treatment (mg/kg, i.p.)	Ultrasonic vocalization time (s)
Vehicle	Vehicle	162.6 ± 16.0 [c]
Vehicle	Ipsapirone (3)	23.4 ± 15.7 [a]
WAY-100635 (0.1)	Vehicle	163.1 ± 9.7 [c]
WAY-100635 (0.1)	Ipsapirone (3)	88.8 ± 16.6 [b,d]
Vehicle	Vehicle	165.2 ± 13.0 [c]
Vehicle	Ipsapirone (3)	19.3 ± 19.6 [a]
WAY-100635 (0.3)	Vehicle	171.5 ± 18.1 [c]
WAY-100635 (0.3)	Ipsapirone (3)	110.0 ± 28.9 [d]

Rats were pre-treated 15 min before treatment with ipsapirone and 15 min later duration of foot shock-evoked ultrasonic vocalization was measured during a 5 min test ($n = 9$–10 per group). Values are the mean ± S.E.M. [a] $P < 0.001$, [b] $P < 0.01$ as compared with vehicle/vehicle control; [c] $P < 0.001$, [d] $P < 0.02$ as compared with vehicle/ipsapirone.

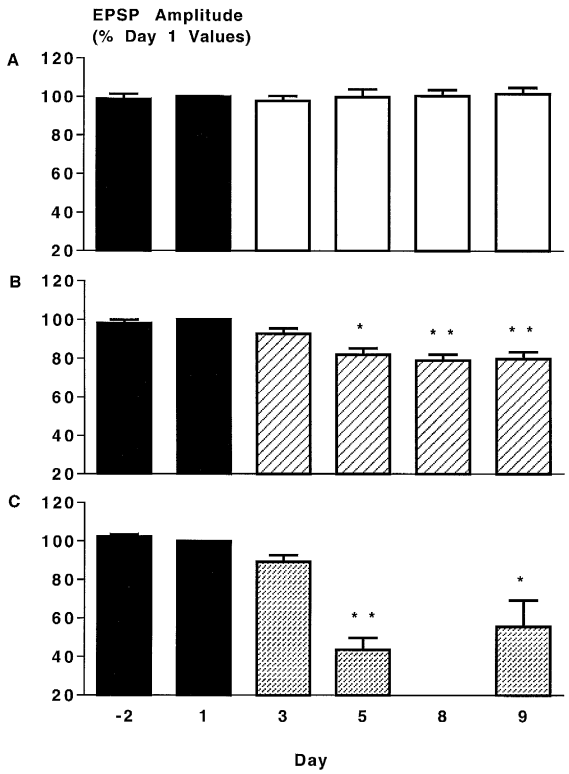

Fig. 3. Subchronic administration of ipsapirone produced a delayed sustained reduction in excitatory synaptic transmission in the hippocampus of freely behaving rats. (A) Repeated administration of water (1 ml/kg per day, i.p., $n = 4$) for 7 days had no effect on the amplitude of the baseline e.p.s.p. measured 24 h after each injection. (B, C) Repeated single daily injections of ipsapirone produced a dose-dependent (B, 1 mg/kg per day for 7 days, i.p., $n = 10$; C, 2 mg/kg per day for 8 days, i.p., $n = 4$) reduction in the amplitude of the baseline e.p.s.p. measured 24 h after each injection. Values are the mean ± S.E.M. * $P < 0.05$, ** $P < 0.01$ compared to water.

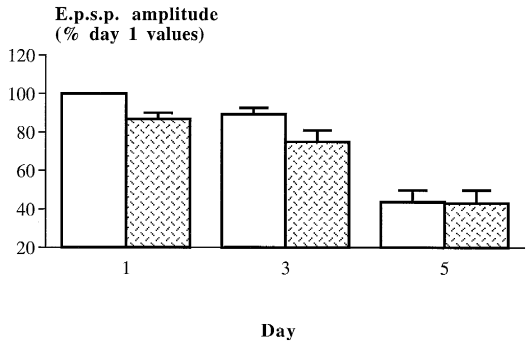

Fig. 4. Acute depressant effect of ipsapirone during subchronic treatment (2 mg/kg per day, i.p.). The gradual reduction in the 24 h baseline (clear bars) e.p.s.p. amplitude was accompanied by a loss of the acute response to the daily injection (stippled bars) by day 5. Values are the mean ± S.E.M.

e.p.s.p. amplitude had decreased, non-significantly, to 92.9 ± 2.8 ($P > 0.05$, compared to 97.9 ± 2.7% in water-injected controls, $n = 4$). The baseline reduction in the field e.p.s.p. amplitude appeared to reach a minimum by about day 5 (82.1 ± 3.5%, $P < 0.05$, versus 99.8 ± 4.6% in controls; $P > 0.05$ compared to 79 ± 3.3% on day 8). On day 9, 2 days after stopping treatment with ipsapirone, there was little evidence for recovery (79.6 ± 4.7%, $P > 0.05$, $n = 8$, compared to ipsapirone on day 8; $P < 0.05$ compared to 101.6 ± 3.8% in water-injected controls, $n = 4$).

Eight days treatment with 2 mg/kg ipsapirone ($n = 4$) also resulted in a gradual decrease in the 24 h baseline e.p.s.p. amplitude (Fig. 3C, $F(1,6) = 18$, $P < 0.01$, compared to water-injected controls, Fig. 3A). This decline appeared to start after the second injection, there being a non-significant decline to 89.2 ± 3.4% on day 3 ($n = 4$, $P > 0.05$ compared with water-injected animals). The reduction in baseline field e.p.s.p. amplitude appeared to reach a minimum by day 5 (43.7 ± 6.1%, $P < 0.01$ compared to water-injected controls) with no further reduction on day 9 (55.5 ± 13.7%, $P > 0.05$ compared to ipsapirone on day 5; $P < 0.05$ compared to water-injected controls on day 9). After 7 days daily treatment with 2 mg/kg ipsapirone, the rats began to show gross behavioural changes, their locomotion being clearly decreased, rarely moving during the 20 min e.p.s.p. baseline recording period. On day 12, 3 days after the last injection with 2 mg/kg, only one animal showed a full recovery of the e.p.s.p. amplitude to day 1 values, the baselines of the other three remaining near to the day 9 levels.

Although the acute depressant effect of the daily injection with 2 mg/kg ipsapirone appeared to be present on day 3 this reduction was not statistically significant ($P >$ 0.05 compared to pre-injection levels, Fig. 4). There was no acute change in the amplitude of the e.p.s.p. on day 5 immediately after the daily injection of the 2 mg/kg dose, a time when there was a marked reduction of the 24 h baseline reading.

3.6. Acute effect of WAY-100635 on the field e.p.s.p. amplitude after 7 days treatment with either 1 mg/kg ipsapirone or water (1 mg/kg) in the freely behaving rat

On day 8 of the subchronic study there was no change in the field e.p.s.p. amplitude over a 1 h period following the injection with 0.25 mg/kg WAY-100635 in rats which had been treated with 1 ml/kg water (Fig. 5A, $n = 4$, $P > 0.05$, compared with pre-injection or day 1 values). However, in the rats subchronically treated with 1 mg/kg ipsapirone there was a clear transient recovery of the field e.p.s.p. amplitude beginning at approximately 25 min and lasting about a further 20 min after administration of WAY-100635 on day 8 ($n = 5$, $P < 0.01$, compared to pre-injection values, Fig. 5B). Indeed, a complete recovery to day 1 values was apparent 35 min after the injection. There were no obvious changes in the locomotor activity of the rats during recording.

3.7. Effect of repeated ipsapirone treatment on ultrasonic vocalizations – relationship to changes in e.p.s.p. baseline

Repeated foot shock over a control period of 8 days produced a relatively stable level of ultrasonic vocalization, measured immediately after the shock, without affecting the baseline e.p.s.p. amplitude, measured 24 h later (Fig. 6). After commencing treatment with ipsapirone (1 mg/kg per day, i.p., $n = 4$) there was a gradual and

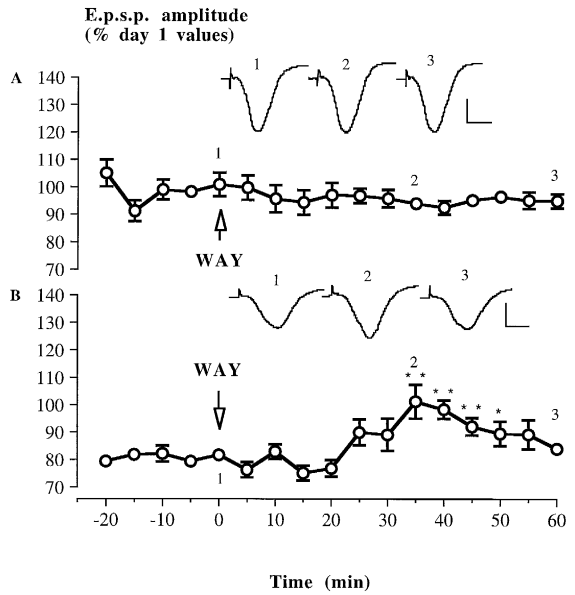

Fig. 5. Effect of the 5-HT_{1A} receptor antagonist WAY-100635 in rats injected repeatedly with either (A) water or (B) ipsapirone (1 mg/kg per day, i.p.) for 7 days. The acute effect of WAY-100635 (WAY, arrow, 0.25 mg/kg, i.p.) on the amplitude of the e.p.s.p. was determined 24 h after the last injection. Values are the mean ± S.E.M. ** $P < 0.01$ compared to pre-injection baseline. Insets show typical traces of the field e.p.s.p. at the times indicated by the numbers. Vertical calibration bar: 1 mV; horizontal calibration bar: 5 ms.

sustained reduction in the amount of time spent vocalizing which paralleled the reduction in excitatory synaptic transmission in the hippocampus. The depressant effect on the vocalizations 24 h after the daily ipsapirone dose became clear after the third day when there was a marked decline ($7.2 \pm 3.1\%$ time vocalizing over a 3 min period, $P < 0.05$ compared to $52.4 \pm 5.8\%$ on day 1). This corresponded to the time when there was a relatively large decrease in the 24 h baseline e.p.s.p. amplitude. Both measures remained reduced for the duration of drug treatment. Four days after ceasing ipsapirone treatment there was only a partial recovery of both measures towards pre-treatment levels. Thus, there was a strong positive correlation between these two measures in the same animals over the period of drug administration and recovery ($r = 0.91$, $P < 0.01$).

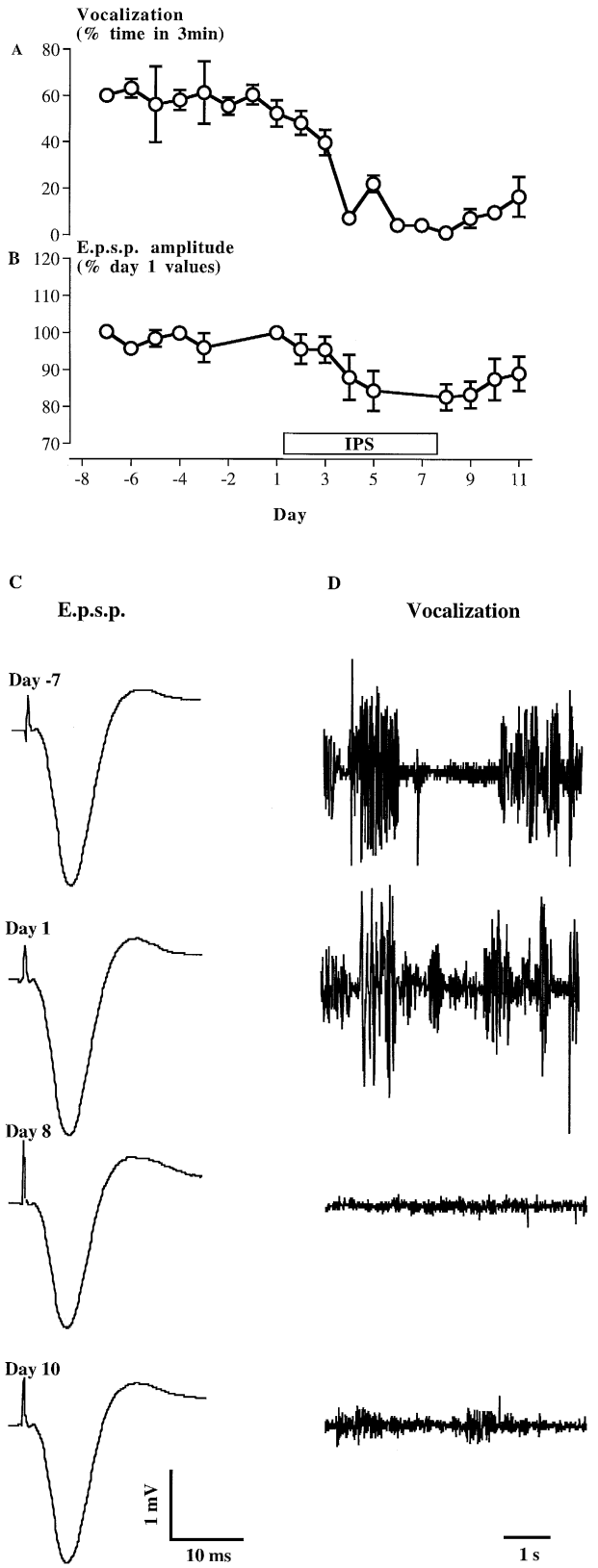

Fig. 6. Comparison of the effect of repeated treatment with ipsapirone on (A) duration of ultrasonic vocalization evoked by foot shock and (B) the amplitude of the e.p.s.p. Control measurements over a period of 7 days (days −7 to day 1) revealed that stable levels of vocalization were accompanied by stable e.p.s.ps. The daily injection of ipsapirone (1 mg/kg per day, i.p.) for 7 days (days 1–7, bar) resulted in a gradual reduction in the duration of ultrasonic vocalizations and a parallel decrease in the baseline e.p.s.p. amplitude. Four days after ceasing ipsapirone treatment (day 11) there was only partial recovery to pre-ipsapirone levels for both measures. Values are the mean ± S.E.M. Insets show sample tracings of recordings of the e.p.s.p. (C) and vocalization (D) for before (days −7 and day 1) and after (days 8 and 10) repeated ipsapirone treatment.

4. Discussion

This study extends our previous finding that repeated treatment with 5-HT_{1A} receptor ligands produces a sustained decrease in excitatory synaptic transmission in the hippocampus of alert, lightly restrained rats, by showing that ipsapirone induced a similar effect in freely behaving animals which was reversed by the highly selective 5-HT_{1A} receptor antagonist WAY-100635. Furthermore, the time course of this depressant effect was very similar to that of an ipsapirone-induced sustained reduction in the aversive response to foot shock.

Acute systemic injection with ipsapirone produced a transient reduction of the e.p.s.p. in the CA1 region of the stratum radiatum in the dorsal hippocampus of freely behaving rats. This is consistent with our previous report that direct intra-hippocampal administration of ipsapirone in alert, lightly restrained animals, mimicked the depressant effect of 5-HT and a number of other 5-HT_{1A} receptor ligands (O'Connor et al., 1990). The present finding of a block of the acute depressant effect of ipsapirone with the highly selective antagonist WAY-100635 is strong evidence that the reduction was due to 5-HT_{1A} receptor activation. Furthermore, pretreatment with a dose of PCPA which previously has been shown to deplete 5-HT in the hippocampus (e.g., Schreiber et al., 1994) did not affect the ipsapirone-induced decrease in e.p.s.p. size. It is thus probable that systemic ipsapirone-induced reduction of

excitatory synaptic transmission was the result of a direct action within the hippocampus at 5-HT_{1A} receptors.

Ipsapirone had a potency which was of the same order of magnitude to that previously found for buspirone and gepirone under somewhat similar conditions (O'Connor et al., 1989; Manahan-Vaughan et al., 1994b). It is unlikely that the shared metabolite 1-pyrimidinylpiperazine (1-PP) made a major contribution to the observed decrease since although it is approximately twice as potent as ipsapirone in reducing hippocampal e.p.s.p. amplitude when injected i.p. (Manahan-Vaughan et al., 1995), its brain levels are approximately one-tenth that of ipsapirone 15–30 min after ipsapirone injection (Nocon et al., 1990).

Whereas the acute effect of a single dose (1 or 2 mg/kg) of ipsapirone was completely reversed within 30 min of injection, repeated treatment for 7–8 days with the same daily dose gradually produced a more marked inhibition which persisted for several days after ceasing treatment. The effect of the 1 mg/kg dose had a very similar time course but was greater in extent (a reduction of 21%) than that of repeated gepirone treatment (13%, 1 mg/kg i.p.; Manahan-Vaughan et al., 1994b). However, the decrease was less than that observed after repeated administration of buspirone at the lower dose of 0.5 mg/kg i.p. (35%, O'Connor et al., 1989). The maximum decrease in the baseline e.p.s.p. amplitude produced by 2 mg/kg ipsapirone was relatively large (56%) and was accompanied by a clear loss of the acute response to the daily injection of ipsapirone. This indicates that the subchronic and acute electrophysiological response to the drug shared a common mechanism. Activation of 5-HT_{1A} receptors is the likely mechanism for this sustained effect because the decrease was fully reversed by WAY-100635.

One possible explanation for the gradual decline in baseline transmission is that repeated ipsapirone injections may have led to a delayed desensitization of somatodendritic 5-HT_{1A} autoreceptors (for extensive discussion, see De Vry, 1995). Previous research has found that subchronic treatment with ipsapirone (5 mg/kg, i.p. twice daily for 14 days) produced a decrease in the electrophysiological responsiveness of somatodendritic 5-HT_{1A} autoreceptors in the dorsal raphe nucleus but no change in receptor number/affinity (Schechter et al., 1990). Longer duration treatment with a higher dose (10 mg/kg, i.p. twice daily for 21 days) did however decrease receptor binding in this nucleus as assessed with quantitative autoradiography (Fanelli and McMonagle-Strucko, 1992). Any decrease in the tonic activation of these receptors should cause an increase in basal 5-HT release/turnover in areas of the brain which receive an input from this nucleus such as the CA1 region of the dorsal hippocampus (Mamounas et al., 1991). Although no change has been detected in the hippocampus (Golembiowska, 1992; Sharp et al., 1993; Schechter et al., 1990) it is possible that small regional changes would go undetected (see Bohmaker et al., 1993). The present finding that PCPA treatment did not affect basal hippocampal transmission in the freely behaving rat may be taken to indicate that the basal levels of 5-HT are not sufficient to tonically activate hippocampal 5-HT_{1A} receptors under our control recording conditions. A similar experiment was attempted in rats which had received repeated ipsapirone injections. However, the results of this study could not be accurately interpreted since the animals became behaviourally agitated on co-administration of PCPA (unpublished observations). Alternative mechanisms for the apparent functional sensitization of hippocampal 5-HT_{1A} receptors after subchronic treatment with ipsapirone might include altered pharmacokinetics (such as drug accumulation) or increased postsynaptic responsiveness. However, thus far the experimental evidence does not support either of these suggestions (Nocon et al., 1990; Schechter et al., 1990; Varrault et al., 1991; Newman et al., 1992; Fanelli and McMonagle-Strucko, 1992).

Ipsapirone appeared to exert a relatively selective anti-aversive effect at the dose range used in these experiments. Neither acute nor repeated treatment with 1 mg/kg ipsapirone had any obvious effect on the general motor activity of the rats during the electrophysiological recording periods. Similar results were obtained for the higher dose of 2 mg/kg, with the exception of a reduction in locomotion on the last 2 days of subchronic treatment. The change in baseline synaptic transmission, however, preceded the decrease in locomotor activity by several days. Although the acute decrease in shock-induced ultrasonic vocalization produced by ipsapirone was obtained at doses similar to those which were effective in reducing the e.p.s.p., and the effects of ipsapirone in both paradigms were attenuated by a similar dose range of WAY-100635, it is possible that both effects involve a different population of 5-HT_{1A} receptors. There is conflicting evidence regarding which brain region is the most important site of action for the acute anxiolytic effect of ipsapirone. Based on currently available data, it seems likely that the hippocampus is a primary target region for the acute anxiolytic-like action of ipsapirone in some behavioural models (e.g., Przegalinski et al., 1994) but not others (e.g., Cervo and Samanin, 1995; for general discussion, see De Vry, 1995). Even though acute intra-hippocampal injection of ipsapirone can lead to a reduction in the duration of ultrasonic vocalizations, this effect may be due to diffusion to more sensitive regions, such as the dorsal raphe nucleus (Schreiber and De Vry, 1993; Jolas et al., 1995). Indeed, the acute anti-aversive effects of the prototypical 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-amino)tetralin (8-OH-DPAT) in this behavioural test have recently been reported to be critically dependent on activation of somatodendritic 5-HT_{1A} receptors in the raphe nuclei (Maurel Remy et al., 1996).

In the subacute study there was a close parallel between the onset, maintenance and recovery of the e.p.s.p.-depressant and anti-aversive effects of ipsapirone in the same animals. Previously, the depressant effect of ipsapirone on

ultrasonic vocalizations was found not to change with repeated treatment (six single injections of 1 mg/kg, i.p. at 3- to 4-day intervals, De Vry et al., 1993, twice-daily injections of 0.1–10 mg/kg, i.p. for 14 days or 10 mg/kg per day, s.c. by minipump for 14 days, De Vry and Schreiber, 1993). However, the possibility of a sustained effect was not examined in detail in these experiments. It is unlikely that the present finding of a long-lasting effect of ipsapirone is due to an accumulation of the drug or some active metabolite (Nocon et al., 1990). The present findings indicate that an adaptive change similar to that found in the electrophysiological experiments may be responsible. Clearly, it is not possible to deduce a causal connection between the sustained reduction in basal excitatory synaptic transmission in the hippocampus and the decrease in ultrasonic vocalizations since the present data are purely correlational. However, the high positive correlation is strongly suggestive of such a relationship. Thus, in contrast to the acute anti-aversive effect of ipsapirone in the ultrasonic vocalization test which may depend primarily on agonist activity in the raphe nuclei (see discussion above), the sustained effect of repeated treatment may be due to activation of postsynaptic 5-HT_{1A} receptors in areas such as the hippocampus, possibly aided by the development of a desensitization of raphe autoreceptors. It should also be noted that there is evidence of an increase in the anti-aversive effect of ipsapirone in the ultrasonic vocalization test to repeated intra-hippocampal but not following intra-raphe injections (four applications over 4 days; De Vry and Schreiber, 1993).

In conclusion, ipsapirone shared the ability of buspirone and gepirone to reduce excitatory synaptic transmission in the hippocampus of alert rats and this effect was enhanced and prolonged following repeated treatment. Furthermore, the latter phenomenon was accompanied by the development of a sustained anti-aversive effect in an ultrasonic vocalization anxiety model. Thus the gradual onset, sustained depression of hippocampal transmission may correspond to and account for the delayed therapeutic effects of these compounds in stress-related psychiatric disorders.

Acknowledgements

Supported by the Health Research Board of Ireland and the Wellcome Trust. The excellent technical assistance of M. Dalmus is gratefully acknowledged (acute ultrasonic vocalization test).

References

Andrade, R. and R.A. Nicoll, 1987, Novel anxiolytics discriminate between postsynaptic serotonin receptors mediating different physiological responses on single neurons of the rat hippocampus, Arch. Pharm. 336, 5.

Blier, P. and C. De Montigny, 1987, Modification of 5-HT neuron properties by sustained administration of the 5-HT_{1A} agonist gepirone: electrophysiological studies in the rat brain, Synapse 1, 470.

Bohmaker, K., A.S. Eison, F.D. Yocca and E. Miller, 1993, Comparative effects of chronic 8-OH-DPAT, gepirone and ipsapirone treatment on the sensitivity of somatodendritic 5-HT_{1A} autoreceptors, Neuropharmacology 32, 527.

Cervo, L. and R. Samanin, 1995, Presynaptic 5-HT_{1A} receptors mediate the effect of ipsapirone on punished responding in rats, Eur. J. Pharmacol. 284, 249.

Colino, A. and J.V. Halliwell, 1987, Differential modulation of three separate K-conductances in hippocampal CA1 neurons by serotonin, Nature 328, 73.

Coop, C.F. and N. McNaughton, 1991, Buspirone affects hippocampal rhythmical slow activity through serotonin$_{1A}$ rather than dopamine D_2 receptors, Neuroscience 40, 169.

Cullen, W.K. and M.J. Rowan, 1994, Gepirone and 1-2-pyrimidinylpiperazine-induced reduction of aversively evoked ultrasonic vocalisation in the rat, Pharmacol. Biochem. Behav. 48, 301.

De Vry, J., 1995, 5-HT_{1A} receptor agonists: recent developments and controversial issues, Psychopharmacology 121, 1.

De Vry, J. and R. Schreiber, 1993, Comparison of acute and repeated treatment with the 5-HT_{1A} receptor ligands 8-OH-DPAT and ipsapirone in animal models of anxiety and depression, Drug Dev. Res. 30, 91.

De Vry, J., U. Benz, R. Schreiber and J. Traber, 1993, Shock-induced ultrasonic vocalization in young adult rats: a model for testing putative anti-anxiety drugs, Eur. J. Pharmacol. 249, 331.

Fanelli, R.J. and K. McMonagle-Strucko, 1992, Alteration of 5-HT_{1A} receptor binding sites following chronic treatment with ipsapirone measured by quantitative autoradiography, Synapse 12, 75.

Forster, E.A., I.A. Cliffe, D.J. Bill, G.M. Dover, D. Jones, Y. Reilly and A. Fletcher, 1995, A pharmacological profile of the selective silent 5-HT_{1A} receptor antagonist WAY-100635, Eur. J. Pharmacol. 281, 81.

Glitz, D.A. and R. Pohl, 1991, 5-HT_{1A} partial agonists. What is their future? Drugs 41, 11.

Godbout, R., Y. Chaput, P. Blier and C. De Montigny, 1991, Tandospirone and its metabolite, 1-(2-pyrimidinyl)-poerazine. I. Effects of acute and long-term tandospirone on serotonin neurotransmission, Neuropharmacology 30, 679.

Golembiowska, K., 1992, Effect of acute and chronic treatment of rats with the putative anxiolytic drug ipsapirone on the turnover of monoamine transmitters in various brain regions. A comparison with the 5-HT_{1A} agonist 8-OH-DPAT, Pol. J. Pharmacol. Pharm. 44, 15.

Harto, N.E., R.J. Branconnier, K.F. Spera and E.C. Dessain, 1988, Clinical profile of gepirone, a nonbenzodiazepine anxiolytic, Psychopharmacol. Bull. 24, 154.

Hirose, A., R. Tsuji, H. Shimizu, T. Tatsuno, H. Tanaka, Y. Kumasaka and M. Nakumura, 1990, Inhibition by 8-hydroxy-2-di-*n*-propylaminotetralin and SM-3997, a novel anxiolytic drug, of the hippocampal rhythmical slow activity mediated by 5-hydroxytryptamine$_{1A}$ receptors, Arch. Pharmacol. 341, 8.

Jolas, T., R. Schreiber, A.M. Laporte, M. Chastanet, J. De Vry, T. Glaser, J. Adrien and M. Hamon, 1995, Are postsynaptic 5-HT_{1A} receptors involved in the anxiolytic effects of 5-HT_{1A} receptor agonists and in their inhibitory effects on the firing of serotonergic neurons in the rat? J. Pharmacol. Exp. Ther. 272, 920.

Leung, L.S., 1979, Orthodromic activation of hippocampal CA1 region of the rat, Brain Res. 176, 49.

Mamounas, L.A., C.A. Mullen, E. O'Hearn and M.E. Molliver, 1991, Dual serotoninergic projections to forebrain in the rat: morphologically distinct 5-HT axon terminals exhibit differential vulnerability to neurotoxic amphetamine derivatives, J. Comp. Neurol. 314, 558.

Manahan-Vaughan, D., R. Anwyl and M.J. Rowan, 1994a, Adaptive changes in 5-HT_{1A} receptor-mediated hippocampal inhibition in the alert rat produced by repeated 8-OH-DPAT treatment, Br. J. Pharmacol. 112, 1083.

Manahan-Vaughan, D., R. Anwyl and M.J. Rowan, 1994b, 5-HT_{1A} receptor-mediated inhibition in the hippocampus of the alert rat – effects of repeated gepirone treatment, Eur. J. Pharmacol. 260, 149.

Manahan-Vaughan, D., R. Anwyl and M.J. Rowan, 1995, The azapirone metabolite 1-2-pyrimidinyl piperazine depresses excitatory synaptic transmission in the hippocampus of the alert rat via 5-HT_{1A} receptors, Eur. J. Pharmacol. 294, 617.

Maurel Remy, S., R. Schreiber, M. Dalmus and J. De Vry, 1996, Somatodendritic 5-HT_{1A} receptors are critically involved in the anxiolytic effects of 8-OH-DPAT, Psychopharmacology 125, 89.

Newman, M.E., B. Shapiro and B. Lerer, 1992, Regulation of 5-hydroxytryptamine$_{1A}$ receptor function in rat hippocampus by short- and long-term administration of 5-hydroxytryptamine$_{1A}$ agonists and antidepressants, J. Pharmacol. Exp. Ther. 260, 16.

Nocon, H., W. Daniel, L. Danek and M. Melzacka, 1990, Cerebral pharmacokinetics of ipsapirone in rats after different routes of administration, J. Pharm. Pharmacol. 42, 642.

O'Connor, J.J., M.J. Rowan and R. Anwyl, 1989, Serotonergic involvement in the inhibitory effects of repeated buspirone treatment on synaptic transmission in the hippocampus, Eur. J. Pharmacol. 167, 21.

O'Connor, J.J., M.J. Rowan and R. Anwyl, 1990, Actions of 5-HT_{1A} receptor ligands on excitatory synaptic transmission in the hippocampus of alert rats, Br. J. Pharmacol. 101, 171.

Przegalinski, E., E. Tatarczynska, A. Klodzinska and E. Chojnacka-Wojcik, 1994, The role of postsynaptic 5-HT_{1A} receptors in the anticonflict effect of ipsapirone, Neuropharmacology 33, 1109.

Schechter, L.E., F.J. Bolanos, H. Gozlan, L. Lanfumey, S. Haj-Dahmane, A.-M. Laporte, C.-M. Fattaccini and M. Hamon, 1990, Alterations in central serotonergic and dopaminergic neurotransmission in rats chronically treated with ipsapirone: biochemical and electrophysiological studies, J. Pharmacol. Ther. 255, 1335.

Schmitz, D., R.M. Empson and U. Heinemann, 1995, Serotonin and 8-OH-DPAT reduce excitatory transmission in rat hippocampal area CA1 via reduction in presumed presynaptic Ca^{2+} entry, Brain Res. 701, 249.

Schreiber, R. and J. De Vry, 1993, Neuronal circuits involved in the anxiolytic effects of the 5-HT_{1A} receptor agonist 8-OH-DPAT, ipsapirone and buspirone in the rat, Eur. J. Pharmacol. 249, 341.

Schreiber, R., M. Brocco, A. Gobert, S. Veiga and M.J. Millan, 1994, The potent activity of the 5-HT_{1A} receptor agonist, S 14506 and S 14671, in the forced swim test is blocked by novel 5-HT_{1A} receptor antagonists, Eur. J. Pharmacol. 271, 537.

Sharp, T., R. Mcquade, S. Bramwell and S. Hjorth, 1993, Effect of acute and repeated administration of 5-HT_{1A} receptor agonists on 5-HT release in rat brain in vivo, Naunyn-Schmiedeberg's Arch. Pharmacol. 348, 339.

Sprouse, J.S. and G.K. Aghajanian, 1988, Responses of hippocampal pyramidal cells to putative serotonin 5-HT_{1A} and 5-HT_{1B} agonists: a comparative study with dorsal raphe neurons, Neuropharmacology 27, 707.

Varrault, A., V. Leviel and J. Bockaert, 1991, 5-HT_{1A}-sensitive adenylyl cyclase of rodent hippocampal neurons: effects of antidepressant treatment and chronic stimulation with agonists, J. Pharmacol. Exp. Ther. 257, 433.

Zhu, X.-O. and N. McNaughton, 1991, Effects of long-term administration of anxiolytics on reticular-elicited hippocampal rhythmical slow activity, Neuropharmacology 30, 1095.