Psychopharmacology (2003) 166:284–293
DOI 10.1007/s00213-002-1339-0

ORIGINAL INVESTIGATION

R. H. McAllister-Williams · A. E. Massey

EEG effects of buspirone and pindolol: a method of examining 5-HT_{1A} receptor function in humans

Received: 10 July 2002 / Accepted: 5 November 2002 / Published online: 13 February 2003
© Springer-Verlag 2003

Abstract *Rationale:* An involvement of 5-HT_{1A} receptors is postulated in the pathophysiology of affective disorders and mechanism of action of antidepressants. Methods for studying their functional integrity in humans are, however, limited. Preliminary data suggests that activation of somatodendritic 5-HT_{1A} receptors cause a negative shift in the EEG frequency spectrum. Animal research suggests that pindolol is an agonist at these receptors but an antagonist at postsynaptic 5-HT_{1A} receptors. *Objective:* We postulated that while pindolol would antagonise known postsynaptic mediated neuroendocrine responses to the 5-HT_{1A} agonist buspirone, both drugs would have a similar effect on the EEG frequency spectrum. *Methods:* Fourteen healthy men were administered placebo or pindolol (20 mg orally) 90 min before placebo or buspirone (30 mg orally) in a double blind cross-over study. Plasma prolactin and growth hormone were assayed and EEGs recorded before and after drug administration. *Results:* A significant negative shift in the EEG frequency spectrum was found for both buspirone and pindolol, with the combination producing a similar effect to each drug alone. In contrast, the neuroendocrine response to buspirone was significantly attenuated by pindolol. *Conclusions:* The data obtained are consistent with the EEG effects of buspirone and pindolol being mediated by somatodendritic 5-HT_{1A} receptors, in contrast to the neuroendocrine response, which is known to be mediated by postsynaptic receptors. The development of this novel method of assessing somatodendritic 5-HT_{1A} receptors in humans is a potentially important advance which may allow the testing of hypotheses of its involvement in depression and response to antidepressants.

Keywords Buspirone · Pindolol · 5-HT · receptors · Prolactin · Growth hormone · Body temperature · EEG

R. H. McAllister-Williams · A. E. Massey
Psychobiology Research Group, School of Neurology, Neurobiology and Psychiatry, University of Newcastle, Newcastle upon Tyne, NE1 4LP, UK

R. H. McAllister-Williams (✉)
Department of Psychiatry, Leazes Wing, Royal Victoria Infirmary, Newcastle upon Tyne, NE1 4LP, UK
e-mail: r.h.mcallister-williams@ncl.ac.uk
Tel.: +44-191-2325131
Fax: +44-191-2275108

Introduction

Serotonergic 5-HT_{1A} receptors are located both presynaptically on serotonin containing raphe neurones (somatodendritic autoreceptors) and postsynaptically on cells throughout the forebrain (Burnet et al. 1995; Azmitia et al. 1996; Pasqualetti et al. 1996). Activation of somatodendritic 5-HT_{1A} autoreceptors plays a key role in determining the activity of the entire serotonergic system (Aghajanian and VanderMaelen 1982) and consequently on the release of 5-HT in terminal areas (Sharp et al. 1993).

Studies investigating the neuroendocrine response to L-tryptophan have shown that depressed patients have an impairment in postsynaptic 5-HT_{1A} receptor function (Charney et al. 1984; Koyama and Meltzer 1986; Cowen and Charig 1987; Deakin et al. 1990; Price et al. 1991), with this impairment being state dependent (Upadhyaya et al. 1991). More recently, positron emission tomography (PET) studies have demonstrated a significant decrease in the number of 5-HT_{1A} receptor sites, both postsynaptically and somatodendritically, in depressed patients (Drevets et al. 2000; Sargent et al. 2000) in line with post-mortem findings (Arango et al. 2001).

5-HT_{1A} receptors may also be central to the mechanism of action of antidepressants (Blier and de Montigny 1994; McAllister-Williams and Young 1998). In vivo studies in rodents have demonstrated that both electroconvulsive shocks and a range of antidepressants when given chronically over 14 days attenuate the function of somatodendritic 5-HT_{1A} receptors located on raphé neurones (Goodwin et al. 1985; Maj and Moryl 1992).

Given the potential importance of 5-HT_{1A} receptors in the pathophysiology of depression and mechanism of

action of antidepressants, it is important to be able to investigate their function in vivo in humans. Currently there are only limited indices for determining this receptor's functional status. The growth hormone response to L-tryptophan, which is abnormal in depressed patients, is believed to be an indicator of postsynaptic (hypothalamic) 5-HT_{1A} function (Smith et al. 1991). A possible alternative assessment of postsynaptic 5-HT_{1A} receptor function is the effect of 5-HT_{1A} agonists on rapid eye movement (REM) sleep. Ipsapirone causes a prolongation of rapid eye movement (REM) latency and decreased total REM time during sleep (Gillin et al. 1994). However, a study using this method has demonstrated no difference in the 5-HT_{1A} response in depressed patients compared to controls (Seifritz et al. 1998), and so is at odds with L-tryptophan neuroendocrine studies (Power and Cowen 1992), post mortem findings (Arango et al. 2001) and PET data (Drevets et al. 2000; Sargent et al. 2000). This discrepancy suggests that postsynaptic 5-HT_{1A} receptors in different anatomical locations are differentially affected in depression. Hypothalamic receptors, as indexed by the neuroendocrine response to L-tryptophan, appear to be functionally attenuated, while pontine receptors, that relate to REM effects of 5-HT_{1A} agonists appear not to be. Given these regional differences it is important that techniques to examine postsynaptic 5-HT_{1A} receptors in areas thought to be particularly relevant to depression, such as frontal cortex, are developed.

Direct functional studies of somatodendritic 5-HT_{1A} receptors in the raphé have also proved difficult. For example, 8-OH-DPAT (a selective 5-HT_{1A} agonist) induces a hypothermia in mice that is believed to reflect somatodendritic 5-HT_{1A} receptor function (Martin et al. 1992). Buspirone (a less selective 5-HT_{1A} agonist) likewise induces hypothermia in humans, but this has been argued to involve at least a degree of postsynaptic 5-HT_{1A} receptor activation (Blier et al. 2002). Alternatively, electroencephalography (EEG) recordings have shown that the administration of 5-HT_{1A} receptor agonists causes a negative shift of the EEG frequency spectrum. This has been demonstrated with ipsapirone during sleep (Seifritz et al. 1996) and buspirone in awake subjects (Anderer et al. 1993; McAllister-Williams et al. 1997). Buspirone administration to rats has a similar effect that is mirrored by the more selective 5-HT_{1A} agonist 8-OH-DPAT, but not a dopamine D_2 antagonist (Bogdanov and Bogdanov 1994). On the basis of in vitro animal electrophysiology (McCormick and Wang 1991), this alteration in the frequency spectrum would be predicated to result from a reduction in 5-HT_2 receptor activation and is similar to that seen with the administration of 5-HT_2 antagonists in humans (Dijk et al. 1989). It has therefore been postulated to result from the activation of somatodendritic 5-HT_{1A} autoreceptors, leading to a reduction in 5-HT release onto postsynaptic 5-HT_2 receptors (Seifritz et al. 1996). The effect of 5-HT_{1A} ligands on these measures may provide a method of examining somatodendritic 5-HT_{1A} receptor function in vivo.

The aim of the current study was to study the effect of buspirone and pindolol alone and in combination on the EEG frequency spectrum recorded from healthy male volunteers, and to compare these effects to known postsynaptic 5-HT_{1A}-mediated neuroendocrine responses. While pindolol is an antagonist at postsynaptic 5-HT_{1A} receptors (Corradetti et al. 1998), it appears to be a partial agonist at the somatodendritic location (Clifford et al. 1998). It was therefore postulated that while pindolol would attenuate buspirone neuroendocrine effects, this would not be the case with regard to the buspirone induced shift in the EEG frequency spectrum if this reflects an effect of buspirone on somatodendritic 5-HT_{1A} receptors.

Materials and methods

Fourteen healthy male subjects aged between 18 and 38 years (mean age 23) gave written informed consent to participate in the study, which had been approved by the local ethics committee. All subjects were free of significant past or present physical ill health and were receiving no medication. They were screened to exclude significant current, past or family history of psychiatric illness.

Experimental procedure and medication

Subjects attended the research laboratory at 0830 hours on four occasions at least 1 week apart. Following insertion of an intravenous cannula and EEG electrode placement, subjects were seated in front of a computer monitor. Baseline investigations were completed that included a number of visual analogue scales (VAS), oral temperature, a venous blood sample for neuroendocrine assays, and an EEG recording. Subjects were then administered pindolol 20 mg or placebo orally (t=0 min). One and a half hours later (t=90 min), subjects were administered buspirone 30 mg or placebo orally. Medication was administered in a double blind random order such that all subjects were treated with placebo followed by placebo, placebo followed by buspirone, pindolol followed by placebo and pindolol followed by buspirone. The order of administration of these four sets of treatments was unique for each subject. Three further sets of investigations (VAS, temperature, neuroendocrine assays, EEG recording) were conducted at 30-min intervals following the administration of buspirone or placebo (t=120, 150 and 180 min).

EEG recordings

EEG was recorded from 29 silver/silver chloride electrodes positioned on the scalp using an elasticated cap (Easy Caps, Germany) and sited in accordance with the International 10-20 system (American Electroencephalographic Society 1994). Further electrodes were placed on the right and left mastoid processes. All channels were recorded relative to the left mastoid. Vertical electrooculograms (VEOG) was recorded between electrodes placed on the nazion and electrodes below the centre of each eye. Horizontal EOG (HEOG) was recorded between electrodes placed on the outer canthus of the eyes. EEG and EOG were filtered with a bandpass of 0.1100 Hz and sampled at a rate of 400 points per second. At each recording time point, subjects were instructed to keep their eyes open and to maintain their gaze on a fixation point (red cross) displayed on a computer monitor, remain still and relaxed, and to keep their minds as blank as possible for three 3-min periods with 30-s intervals between. Ten minutes of continuous EEG was acquired during this period.

EEG analysis

A standardised EEG analysis procedure was followed using Neuroscan Scan 4.1 software (Neurosoft Inc., USA). The EEG was initially visually inspected and sections contaminated by gross artefacts rejected. A blink-correction procedure (Semlitsch et al. 1986) was employed to remove the blink EOG artefact from concurrently recorded EEG. Because of concerns of artefacts originating from the electrocardiogram (ECG) contaminating the element of the frequency spectrum of interest (with heart rate also being potentially affected by pindolol), great care was taken to correct any visible artefact. The right mastoid channel was visually inspected for evidence of ECG activity. If any was observed, a principle component analysis (PCA) was conducted to remove the artefact from the active EEG channels. In practice, the maximum deflection seen in the ECG QRS complex in the right mastoid channel needed to be at least ±10–15 μV for the signal-to-noise ratio to be sufficient to allow the PCA to be conducted. Following artefact correction, data was algebraically re-referenced to represent recordings from average mastoid reference and epoched into segments 10.24 s long. Any epoch in which any channel, except VEOG, had a voltage deflection greater than ±75 μV was excluded. Remaining epochs underwent Fast Fourier Transformation (FFT) and were averaged together. The precision of the FFT was 0.098 Hz. Potential shifts in the EEG frequency spectrum were assessed by calculating the centroid frequency (Anderer et al. 1993) which is the mean frequency between two points weighted relative to the spectral power. Frequencies less than around 5–6 Hz are particularly susceptible to influence from recording artefacts and their correction. In addition, previous pilot data suggested buspirone affected the frequency spectrum up to around 10 Hz (McAllister-Williams et al. 1997). As a result, the centroid was calculated between 6 and 10.5 Hz being the composite of theta (6.0–8.5 Hz) and alpha 1 (8.5–10.5 Hz) frequency bands based on factor analysis of previous pharmaco-EEG data (Herrmann et al. 1980). The standardised analysis protocol used led to extremely high inter-rated reliability. Five sets of data contaminated by a representative range of artefacts were analysed independently by the two authors. Centroid frequencies between 6 and 10.5 Hz averaged across electrode sites had a Pearson's correlation coefficient r^2 of 1.000 ($P<0.0001$), with a mean difference of 0.003±0.005 Hz between the two raters.

Due to the large amount of EEG data collected (29 active electrode sites with recordings made at four time points), initial analysis concentrated on EEG activity recorded from a single electrode site (Cz-occiput). Subsequent topographical analysis was conducted on EEG data recorded from all active electrode sites.

Neuroendocrine and other measures

Blood samples were taken into EDTA tubes, centrifuged at 3000 rpm for 10 min and plasma removed and stored at −20°C. Plasma was also ultra-filtered and stored until assay. Samples were analysed for prolactin (PRL) and growth hormone (GH) using radioimmunoassay kits (Immuno Diagnostic Systems Ltd, Boldon, Tyne and Wear, UK). Intra- and inter- assay coefficients of variation for PRL were 5.7% and 6.4%, respectively, and GH 2.7% and 7.4%. GH data obtained on visits by subjects when their baseline was greater than 10 mIU/l were excluded, since GH inhibits its own secretion (Checkley 1980). In addition, some samples were spoilt or not obtainable. Therefore the numbers of subjects' data available for analysis varied for different comparisons. Temperature was assessed using a digital thermometer accurate to 0.1°C placed sublingually. Visual analogue scales (VAS) consisted of 100 mm scales on which subjects rated "depression", "drowsiness", "restlessness", "nausea" and "lightheadedness", with "the most severe possible" at one end and "not at all" at the other.

Statistical analysis

In each element of the study, an ANOVA was conducted including all four treatment arms (SPSS version 9.0, Chicago, Ill., USA). If a main effect of treatment was found, then subsidiary ANOVAs were conducted comparing placebo+placebo (hereafter simply referred to as placebo) with placebo+buspirone (referred to as buspirone); placebo with pindolol+placebo (referred to as pindolol); placebo with pindolol+buspirone; and buspirone with pindolol+buspirone chosen on the basis of the a priori hypotheses. For the analysis of the centroid frequency, the change in centroid over the 90 min following placebo or buspirone administration was calculated as an area under the curve (AUC) for the frequency at t=120, 150 and 180 min relative to t=0 using the trapezoid method for each of the 29 active electrode sites. ANOVA employed within subject factors of "treatment" and "electrode site". Neuroendocrine and temperature data ANOVA employed within subject factors of "treatment" and "time" (0, 120, 150 and 180 min). VAS data was analysed with within subject factors of "treatment", "time" and "VAS scale" (depression, drowsiness, restlessness, nausea and lightheadedness). Subsidiary ANOVAs were conducted for each of the five VASs separately. The Kolmogorov-Smirnov test was applied to all data. This revealed no significant departure from normality of the centroid frequency AUC values, temperature and the PRL data. The spread of the GH data was significantly different from normal, but this was not the case for log transformed data which were used in all analyses. VAS data also deviated from normality as did log transformed values, though less so. Given the number of potential comparisons if a non-parametric test was used, log transformed VAS values were analysed with ANOVA, which is relatively robust with respect to violations of the assumption of normality (Howell 1999). All ANOVA analyses incorporated the Greenhouse-Geisser correction for inhomogeneity of covariance, with F ratios reported with corrected degrees of freedom. All values are quoted as mean±SD.

Results

Subjects

Subjects scored a mean of 0.3±0.8 (mean±SD, range 0–3) on the Hamilton Depression Rating Scale 21 item (HAMD-21) and 1.3±1.5 (range 0–4) on the Beck Depressive Inventory (BDI). Mean full scale IQ was estimated with the National Adult Reading Test (NART) to be 102±9 (range 90–113). Body mass index for the subjects was 24.7±4.6 kg/m^2 (range 19.0–30.8).

EEG effects of buspirone

As previously reported (Anderer et al. 1993; McAllister-Williams et al. 1997), buspirone caused a small negative shift of the EEG frequency spectrum below 10–11 Hz, as illustrated for the Cz electrode in Fig. 1A. This effect was quantified at the three recording time points, t=120, 150 and 180 min (i.e. 30, 60 and 90 min post-buspirone administration) by calculating the centroid frequency between 6 and 10.5 Hz. Figure 2A shows a non-significant decrease in the centroid frequency from 7.80±0.37 to 7.72±0.38 Hz following placebo administration. Buspirone caused a larger negative shift in the centroid frequency with the largest effect being seen at t=180 min when the frequency decreased from

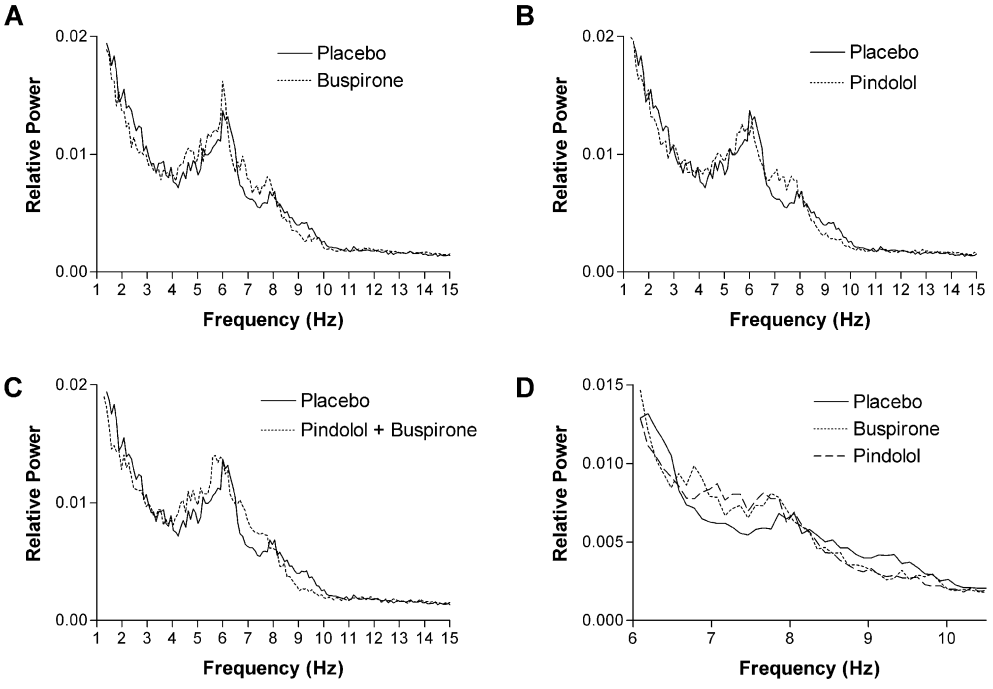

Fig. 1A–D Effect of buspirone and pindolol on the EEG frequency spectra. All data recorded at t=150 min (150 min post-pindolol or placebo and 60 min post-buspirone or placebo administration). **A**–**C** EEG power calculated from FFT absolute power values relative to total power between 0.5 and 30 Hz, plotted to 15 Hz. **D** EEG frequency spectrum plotted between 6 and 10.5 Hz only for placebo, buspirone and pindolol

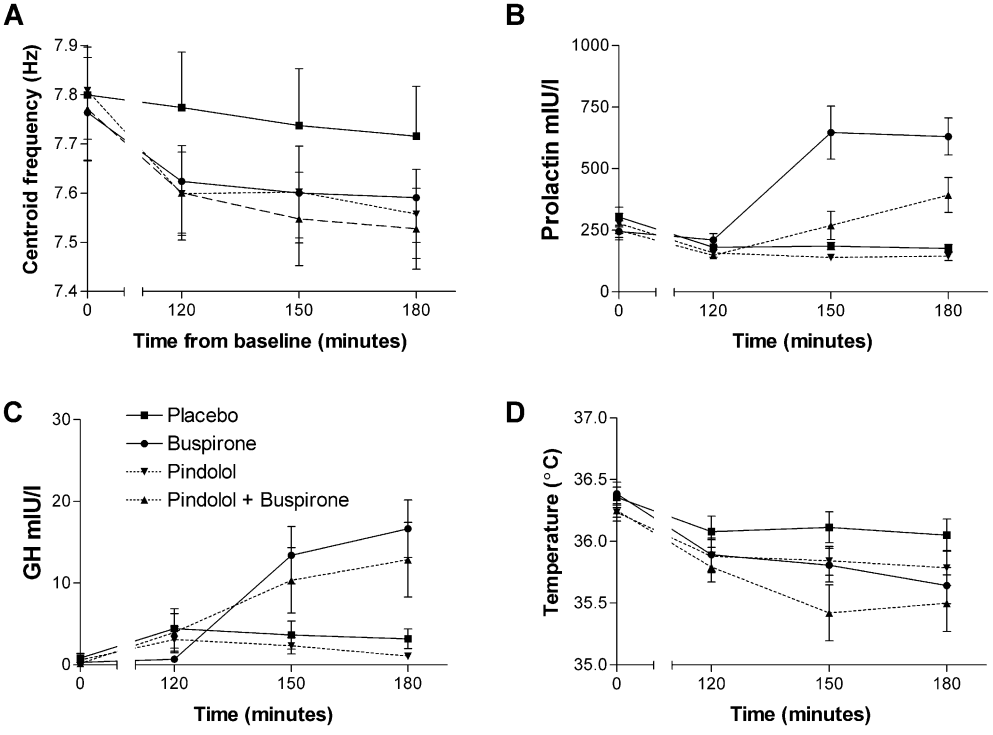

Fig. 2A–D Time course of the effect of buspirone and pindolol. All data plotted for t=0, 120, 150 and 180 min. Note the discontinuous *x*-axis. Times refer to the interval since pindolol or placebo administration. Buspirone or placebo was administered at t=90 min. **A** EEG centroid frequency. Centroid frequency calculated between 6 and 10.5 Hz for Cz electrode site only. **B** Prolactin response. **C** Growth hormone response. **D** Hypothermic response

7.77±0.36 at baseline to 7.59±0.34 Hz (Fig. 2A). To assess possible topographical variations in the effect of buspirone, the change in centroid frequency was calculated as the AUC for the centroid frequency recorded at t=120, 150 and 180 min, relative to the frequency recorded at t=0 min, for all 29 active electrode sites. A representative subset of electrode sites is illustrated in Fig. 3. It can be seen that buspirone caused a larger bilaterally symmetrical reduction in centroid than placebo, with a slightly larger effect seen at posterior compared to anterior electrode sites.

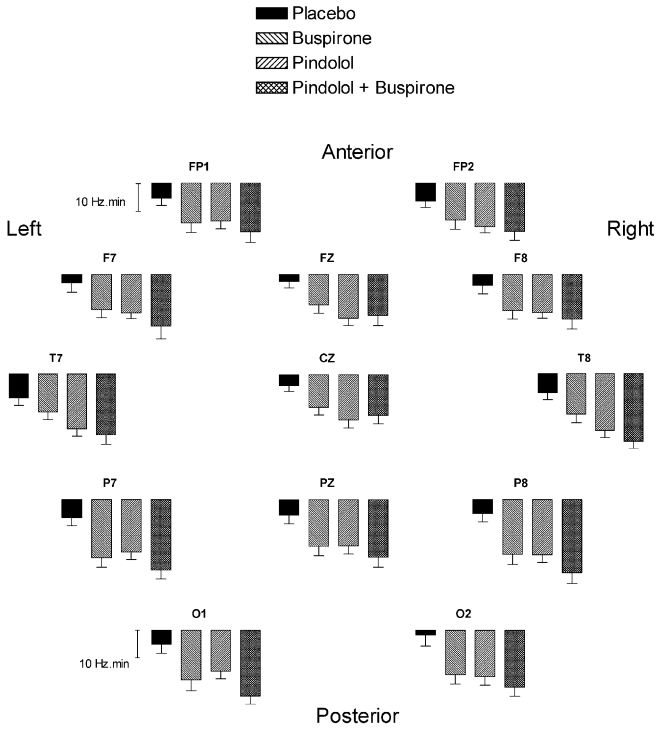

Fig. 3 Topography of the effect of buspirone and pindolol on EEG centroid frequency. Centroid frequencies were calculated between 6 and 10.5 Hz for each of 13 representative EEG electrode sites across the scalp. The change in centroid at t=120, 150 and 180 min relative to t=0 calculated as an area under the curve (*AUC*) is plotted for each electrode position as if looking down on the head from above. Sites are labelled using the extended International 10-20 system (American Electroencephalographic Society 1994)

Effect of pindolol on the EEG frequency spectrum

Figure 1B illustrates that a similar negative shift in the EEG frequency spectrum occurred relative to placebo with pindolol as seen with buspirone. The largest effect of pindolol was also seen at t=180 min, when the centroid frequency was reduced from 7.81±0.37 to 7.56±0.34 Hz (Fig. 2A). Figure 3 illustrates a similar magnitude and topographical distribution of the effect of pindolol on the centroid frequency compared to buspirone.

As postulated, pretreatment with pindolol had no effect on the buspirone EEG effects. Indeed, the combination of pindolol and buspirone produced a similar effect on the EEG frequency spectrum as buspirone and pindolol alone (Fig. 1C). The time course and topography of the effect of pindolol+buspirone was also similar to pindolol and buspirone alone (Figs 2A and 3). Treatment with pindolol+buspirone caused the centroid to change from 7.77±0.39 at baseline to 7.53±0.31 at t=180 min.

Statistical analysis of EEG effects

ANOVA of the AUC of the shift in centroid relative to baseline, with within-subject factors of all four drug treatments and all 29 electrode sites, revealed a highly significant effect of treatment [$F(2.3,29.3)=7.76$, $P<0.001$]. The mean change in centroid across all electrode sites was −4.84 Hz.min with placebo. Post-hoc analysis revealed this to be significantly increased to −14.73 Hz.min by buspirone [$F(1,13)=15.09$, $P<0.005$], −16.68 Hz.min by pindolol [$F(1,13)=10.37$, $P<0.01$] and −19.64 Hz.min by pindolol+buspirone [$F(1,13)=16.29$, $P<0.001$]. There was no significant difference between the shift in centroid with buspirone and pindolol+buspirone treatment [$F(1,13)=2.19$, $P=0.16$].

Possible topographical differences in the effects of buspirone and pindolol on the shift in centroid frequency were examined by selecting two electrodes in each of four quadrants (left anterior–FP1 and F7; right anterior–FP2 and F8; left posterior–P7 and O1; right posterior–P8 and O2). Analysis included within-subject factors of treatment, hemisphere and anterior/posterior location. ANOVA revealed a main effect of drug [$F(2.2,28.2)=9.39$, $P<0.001$] and an anterior/posterior effect [$F(1,13)=8.59$, $P<0.05$] due to the shift in centroid being larger posteriorly (−16.23 Hz.min) compared to anterior sites (−12.45 Hz.min). However there was no drug by hemisphere or drug by anterior/posterior interaction. This was also the case when post-hoc ANOVAs were conducted on pairwise treatment comparison, demonstrating a similar topographical distribution of the effects of buspirone and pindolol.

Neuroendocrine response to buspirone and pindolol

Figure 2B illustrates the PRL responses to the four treatments. ANOVA employing within-subjects factors of treatment and time found a significant main effect of treatment [$F(1.7,16.6)=15.80$, $P<0.001$] and a significant drug by time interaction [$F(2.6,26.3)=10.49$, $P<0.001$]. As expected, buspirone, but not pindolol caused an increase in plasma PRL, as demonstrated by a significant treatment by time interaction for the effect of buspirone relative to placebo [$F(1.6,20.7)=20.09$, $P<0.001$]. In contrast to the EEG effect, pindolol pretreatment significantly decreased the response to buspirone [$F(1.0,11.0)=20.07$, $P<0.001$].

GH responses showed a similar profile (Fig. 2C). ANOVA revealed a treatment by time interaction for all four treatments [$F(4.0,32.3)=5.49$, $P<0.005$]. This resulted from a significant increase in GH following treatment with buspirone relative to placebo [$F(1.0,10.0)=12.61$, $P<0.005$], while the effect of pindolol did not significantly differ from placebo. There was a significant reduction in the GH response to buspirone following pindolol pretreatment, revealed by a significant treatment by time interaction for the comparison of buspirone with pindolol+buspirone [$F(2.3,25.7)=7.64$, $P<0.005$]. In addition, the GH response following pindolol+buspirone was not significantly different from that seen with placebo.

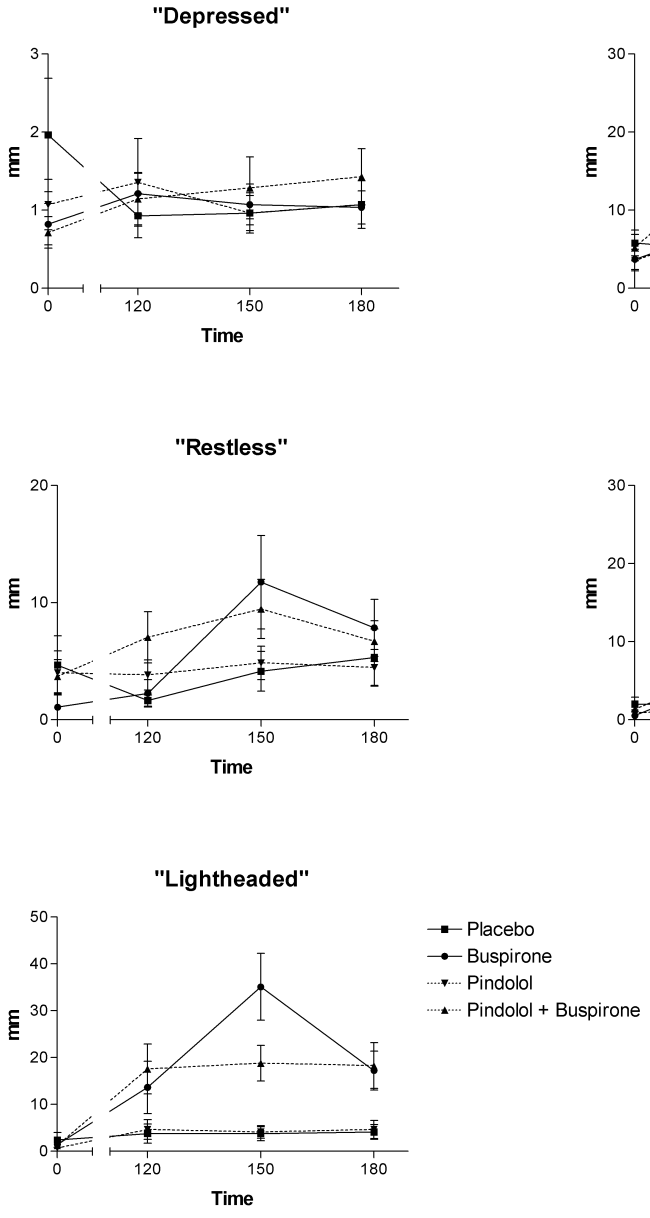

Fig. 4 Effect of buspirone and pindolol on various subjective symptoms rated using a 100 mm visual analogue scale. Details of graph as in Fig. 2

Effect of buspirone and pindolol on body temperature

Analysis of the effect of the four treatments on body temperature (Fig. 2D) revealed a main effect of treatment [$F(2.0,25.4)=6.30$, $P<0.01$]. Post-hoc analysis revealed a main effect of buspirone compared to placebo [$F(1.0,13.0)=7.65$, $P<0.05$]. In line with the EEG effects, but in contrast to the neuroendocrine responses, there was a main effect of pindolol relative to placebo on body temperature [$F(1.0,13.0)=6.67$, $P<0.05$]. In addition, pindolol pretreatment had no significant effect on buspirone induced hypothermia, with the effect of the pindolol+buspirone treatment being highly significantly different from placebo [$F(1.0,13.0)=9.96$, $P<0.005$].

Subjective effects of buspirone and pindolol

Initial analysis of the effects of the four treatments on subjective symptoms as assessed by five VAS involved a grand ANOVA with within subject factors of treatment, time and VAS scale. This revealed a main effect of treatment [$F(2.3,25.2)=6.33$, $P<0.005$] and a treatment by VAS interaction [$F(4.0,43.5)=2.64$, $P<0.05$], implying a non-uniform effect of treatment on the various scales. As a first step in the post-hoc analysis of these data, ANOVAs were conducted on each of the five VAS measures including all four treatments. No significant treatment or treatment by time interactions were found for the "depressed" and "restless" VAS (see Fig. 4A, C) and these were not analysed further. Of the remaining three scales ("drowsy", Fig. 4B; "nausea", Fig. 4D; "lightheaded", Fig. 4E), a similar pattern of effects was found.

Subsidiary post-hoc ANOVAs for each of these scales comparing pairs of treatments showed a general pattern of effects similar to those seen with the neuroendocrine data. A significant main effect of treatment and/or a treatment by time interaction was seen for the comparison of buspirone with placebo [drowsiness: treatment by time $F(1.65,21.5)=7.59$, $P<0.005$; nausea: treatment $F(1.0,13.0)=6.24$, $P<0.05$, treatment by time $F(1.9,25.2)=9.97$, $P<0.001$; lightheadedness: treatment $F(1.0,13.0)=17.67$, $P<0.001$, treatment by time $F(2.1,27.9)=12.60$, $P<0.001$]. Conversely, the comparison of pindolol and placebo revealed no significant effects of treatment or treatment by time interactions for any of the scales, demonstrating a lack of effect of pindolol alone. Pindolol pretreatment, however, did reduce the effects of buspirone on all three scales, as shown by a significant treatment by time interaction comparing buspirone with pindolol+buspirone [drowsiness: $F(2.1,25.2)=4.70$, $P<0.05$; nausea: $F(1.7,20.6)=3.15$, $P<0.05$; lightheadedness: $F(2.7,31.9)=3.09$, $P<0.05$].

Discussion

This study has demonstrated that 5-HT_{1A} ligands cause a significant negative shift of the EEG frequency spectrum, with buspirone 30 mg and pindolol 20 mg having a similar magnitude of effect. There was no evidence of pindolol pretreatment reducing the effect of buspirone on the frequency spectrum. A similar relative effect of buspirone and pindolol was found with regard to the hypothermic effect of these ligands. These findings were in contrast to the GH and PRL neuroendocrine responses and the subjective effects of these drugs. While buspirone administration led to an increase in the plasma concentrations of GH and PRL, and increased drowsiness, nausea and lightheadedness, pindolol alone had little or no effect. Pindolol pretreatment, however, significantly reduces these responses to buspirone. It therefore appears that the relative effects of these two 5-HT_{1A} ligands on the EEG frequency spectrum and body temperature are different to their neuroendocrine and subjective effects. These findings are in line with our a priori hypotheses and are consistent with the shift in EEG centroid and the hypothermic response to buspirone being mediated (at least in part) by somatodendritic 5-HT_{1A} receptors where pindolol acts as a partial agonist (Clifford et al. 1998). In contrast, the neuroendocrine and subjective response to buspirone may result from activation of a 5-HT_{1A} receptor in a different location, where pindolol has little intrinsic activity (Corradetti et al. 1998) but blocks the effects of buspirone.

The PRL and GH responses to buspirone are similar to previous findings (Meltzer et al. 1983; Coccaro et al. 1990; Young et al. 1994; Anderson et al. 1996). Likewise, pindolol has previously been reported to have no effect on PRL or GH plasma concentrations (Anderson and Cowen 1992), though a more recent study has suggested that pindolol may decrease PRL secretion (possibly due to blockade of tonic 5-HT activity (Meltzer and Maes 1996)). Pindolol pretreatment has previously been reported to inhibit the GH response both to buspirone (Anderson and Cowen 1992) and the more potent 5-HT_{1A} agonist, flesinoxan (Seletti et al. 1995). The situation regarding pindolol inhibition of the PRL response to 5-HT_{1A} agonists is not as clear. In line with the current data, a small study has previously shown that pindolol dose dependently inhibits the PRL response to buspirone (Coccaro et al. 1990). However, the PRL response to flesinoxan is unaffected by pindolol pretreatment (Seletti et al. 1995), and Anderson and Cowen (1992) reported that pretreatment with pindolol 30 mg slowed, rather than reduced, the PRL response to buspirone. However, caution needs to be exercised when interpreting the neuroendocrine responses to buspirone, since non-serotonergic mechanisms may be involved. It has been argued that the PRL response in particular may involve a dopaminergic element (Maskall et al. 1995), due to potential dopaminergic antagonistic properties of buspirone (Tunnicliff 1991).

The hypothermic effects of several 5-HT_{1A} agonists in humans have been well reported, including buspirone (Young et al. 1994; Anderson et al. 1996), ipsapirone (Meltzer and Maes 1995; Lerer et al. 1999; Shapira et al. 2000), gepirone (Anderson et al. 1990) and flesinoxan (de Koning and de Vries 1995; Pitchot et al. 1995; Seletti et al. 1995). However the effect of pindolol on body temperature has been much less studied. It has been reported that pindolol alone has no effect (Anderson and Cowen 1992), though another study showed it to induce a hypothermic response as found in the current study (Meltzer and Maes 1996). Contrary to the current findings, pindolol has been reported to inhibit the hypothermic response to buspirone (Anderson and Cowen 1992), ipsapirone (Hennig et al. 1997) and flesinoxan (Seletti et al. 1995), though to a non-significant extent in the latter case. The reasons for the discrepancy with the current study are unclear, but the variability between studies may relate to the small effect size of 5-HT_{1A} ligands on body temperature. These inconsistent findings, together with debate over the relative roles of somatodendritic and postsynaptic 5-HT_{1A} receptors in the hypothermic response (Blier et al. 2002), suggest that it is of limited practical use as an index of serotonergic function.

The use of centroid frequency analysis to assess changes in the EEG frequency spectrum is not novel (e.g. Anderer et al. 1993). However, most previous studies that have measured the centroid frequency of the EEG frequency spectrum have employed FFT analyses with precessions of 0.5 Hz. This requires obtaining 2-s epochs of data. Potentially a greater precision than this is needed when assessing possible subtle interactions between treatments as in this study. However, the cost of an approximate 0.1 Hz precision in the FFT is the necessity to acquire 10-s long epochs of data. This increases the probability of an artefact contaminating any epoch. Artefact handling is therefore of critical importance,

which can depend on subjective decisions taken during the analysis procedure. This study has, however, demonstrated that by employing a strict analysis protocol, inter-rater reliability can be extremely high. The problems of artefact contamination were further reduced by employing the measure of the centroid frequency above 6 Hz, since most physiological artefacts introduce noise into the frequency spectrum below this frequency. However, it is also possible that by restricting our measure only to frequencies above 6 Hz, we may have "lost" some of the "signal" from drug administration. Despite this, buspirone caused a significant reduction in the centroid frequency between 6 and 10.5 Hz, confirming our own and another group's preliminary observations (Anderer et al. 1993; McAllister-Williams et al. 1997) and in line with previous animal studies using more specific 5-HT_{1A} ligands (Bogdanov and Bogdanov 1994).

The EEG recordings obtained in this study involved relatively long periods of time when subjects were asked to keep still and relaxed, with their minds as blank as possible. This introduces a potential weakness into the study, since it is impossible to know exactly what cognitive processes subjects were engaged in during recordings. This is of some concern since cognitive activity may alter either the baseline centroid frequency and/or the effect of 5-HT_{1A} ligands. In this regard, it has been reported that buspirone administration can induce frontal midline theta activity in healthy subjects performing mental arithmetic (Mizuki et al. 1994). We have previously examined the effect of buspirone on the centroid frequency in five subjects, both when resting with their eyes open, as in the current study, and while undertaking a mental arithmetic task. There was no difference in the baseline centroid pre-buspirone administration between the two conditions, nor was there any difference in the magnitude of the effect of buspirone (unpublished observations–data available on request). Despite these findings, it is clearly important that the effect of varying levels of alertness and cognitive function on the EEG centroid frequency is examined.

It is impossible to rule out a pharmacokinetic explanations of our findings. For example the access of buspirone and pindolol to receptors in different locations may vary. An additional possible concern is whether the EEG effects of buspirone and pindolol are simply epiphenomena of their subjective effects. This seems unlikely, given the difference in the relative effects of buspirone and pindolol in causing the subjective symptoms compared to causing a negative shift in the EEG frequency spectrum.

Buspirone and pindolol are not ideal highly selective 5-HT_{1A} receptor ligands. Buspirone is a weak dopamine D_2 antagonist and its major metabolite, 1-PP, is an α_2-adrenoceptor antagonist (Tunnicliff 1991). Pindolol is a potent β-adrenoceptor antagonist. It seems unlikely that the endocrine effects of buspirone are mediated by D_2 or α_2 receptors, since they are blocked by pindolol and the only pharmacological property the drugs have in common is an action at 5-HT_{1A} receptors. However, it is possible, though perhaps unlikely, that both drugs cause a shift in the EEG frequency spectrum of a similar nature via buspirone's action on D_2 or α_2 receptors and pindolol's action on β-adrenoceptors, rather than involving 5-HT_{1A} receptors. Clarification of this point awaits the availability of more selective ligands for use in humans.

While the shift in EEG frequency spectrum by 5-HT_{1A} ligands has a pharmacological profile that would support the effect being mediated by activation of somatodendritic 5-HT_{1A} receptors, the mechanism and locus of the neuronal activity that leads to changes in scalp EEG is unclear. It has previously been reported that the 5-HT_{1A} agonist ipsapirone causes an increase in slow wave EEG activity during sleep (Seifritz et al. 1996), with a similar pattern to that seen with a 5-HT_2 antagonist (Dijk et al. 1989). It has been argued that activation of somatodendritic 5-HT_{1A} receptors leads to a decrease in release of 5-HT onto 5-HT_2 receptors in cortex, producing a similar effect to a 5-HT_2 antagonist on sleep EEGs (Seifritz et al. 1996). If these sleep EEG findings relate to a similar phenomenon to the shift in the EEG frequency spectrum seen in awake subjects, then it is possible that a similar explanation applies. The wide cortical distribution of 5-HT neurones from the raphé is concordant with the generalised effect on the frequency spectrum seen across the scalp. Further work both in animals and humans is required to delineate conclusively the locus of action of 5-HT_{1A} ligands and the mechanism of effect on the EEG frequency spectrum.

The current study provides some interesting results. It is probable that the shift in the EEG frequency spectrum following administration of 5-HT_{1A} agonists is an in vivo index of the function of somatodendritic 5-HT_{1A} receptors. Further work is required to clarify the situation, which would be aided by the use of more specific and potent 5-HT_{1A} agonists and antagonists than buspirone and pindolol. However, the methodology used here will prove to be extremely useful in both the examination of the functional status of somatodendritic 5-HT_{1A} receptors in affective disorders and the mechanism of action of antidepressants and other psychotropics.

Acknowledgements The authors would like to thank Richard Marsh for technical assistance during this project. They would also like to thank Professors I.N. Ferrier and C.H. Ashton for helpful discussions throughout the development of the techniques and Professors I.N. Ferrier, A.H. Young and C.D. Ingram and Dr S. Gartside for helpful comments on the manuscript. This study was funded by means of a Medical Research Council (UK) Clinician Scientist Fellowship award to R.H.McA-W.

References

Aghajanian GK, VanderMaelen CP (1982) Intracellular recordings from serotonergic dorsal raphe neurons: pacemaker potentials and the effect of LSD. Brain Res 238:463–469

American Electroencephalographic Society (1994) Guideline thirteen: guidelines for standard electrode position nomenclature. J Clin Neurophysiol 11:111–113

Anderer P, Barbanoj MJ, Saletu B, Semlitsch HV (1993) Restriction to a limited set of EEG-target variables may lead to

misinterpretation of pharmaco-EEG results. Neuropsychobiology 27:112–116
Anderson IM, Cowen PJ (1992) Effect of pindolol on endocrine and temperature responses to buspirone in healthy volunteers. Psychopharmacology 106:428–432
Anderson IM, Cowen PJ, Grahame-Smith DG (1990) The effects of gepirone on neuroendocrine function and temperature in humans. Psychopharmacology 100:498–503
Anderson IM, Deakin JFW, Miller HE (1996) The effect of chronic fluvoxamine on hormonal and psychological responses to buspirone in normal volunteers. Psychopharmacology 128:74–82
Arango V, Underwood MD, Boldrini M, Tamir H, Kassir SA, Hsiung S, Chen JJ, Mann JJ (2001) Serotonin 1A receptors, serotonin transporter binding and serotonin transporter mRNA expression in the brainstem of depressed suicide victims. Neuropsychopharmacology 25:892–903
Azmitia EC, Gannon PJ, Kheck NM, Whitaker-Azmitia PM (1996) Cellular localization of the 5-HT1A receptor in primate brain neurons and glial cells. Neuropsychopharmacology 14:35–46
Blier P, de Montigny C (1994) Current advances in the treatment of depression. Trends Pharmacol 15:220–226
Blier P, Seletti B, Gilbert F, Young SN, Benkelfat C (2002) Serotonin (1A) receptor activation and hypothermia in humans. Lack of evidence for a presynaptic mediation. Neuropsychopharmacology 27:301–308
Bogdanov NN, Bogdanov MB (1994) The role of 5-HT1A serotonin and D2 dopamine receptors in buspirone effects on cortical electrical activity in rats. Neurosci Lett 177:1–4
Burnet PW, Eastwood SL, Lacey K, Harrison PJ (1995) The distribution of 5-HT1A and 5-HT2A receptor mRNA in human brain. Brain Res 676:157–168
Charney DS, Heninger GR, Sternberg DE (1984) Serotonin function and mechanism of action of antidepressant treatment. Effects of amitriptyline and desipramine. Arch Gen Psychiatry 41:359–365
Checkley SA (1980) Neuroendocrine tests of monoamine function in man: a review of basic theory and its application to the study of depressive illness. Psychol Med 10:35–53
Clifford EM, Gartside SE, Umbers V, Cowen PJ, Hajos M, Sharp T (1998) Electrophysiological and neurochemical evidence that pindolol has agonist properties at the 5-HT(1A) autoreceptor in vivo. Br J Pharmacol 124:206–212
Coccaro EF, Gabriel S, Siever LJ (1990) Buspirone challenge: preliminary evidence for a role for central 5-HT1a receptor function in impulsive aggressive behaviour in humans. Psychopharmacol Bull 26:393–405
Corradetti R, Laaris N, Hanoun N, Laporte AM, Le Poul E, Hamon M, Lanfumey L (1998) Antagonist properties of (–)-pindolol and WAY 100635 at somatodendritic and postsynaptic 5-HT1A receptors in the rat brain. Br J Pharmacol 123:449–462
Cowen PJ, Charig EM (1987) Neuroendocrine responses to intravenous tryptophan in major depression. Arch Gen Psychiatry 44:958–966
de Koning P, de Vries MH (1995) A comparison of the neuroendocrinological and temperature effects of DU 29894, flesinoxan, sulpiride and haloperidol in normal volunteers. Br J Clin Pharmacol 39:7–14
Deakin JFW, Pennell I, Upadhyaya AJ, Lofthouse R (1990) A neuroendocrine study of 5-HT function in depression: evidence for biological mechanisms of endogenous and psychosocial causation. Psychopharmacology 101:85–92
Dijk DJ, Beersma DG, Daan S, van den Hoofdakker RH (1989) Effects of seganserin, a 5-HT2 antagonist, and temazepam on human sleep stages and EEG power spectra. Eur J Pharmacol 171:207–218
Drevets WC, Frank E, Price JC, Kupfer DJ, Greer PJ, Mathis C (2000) Serotonin type-1A receptor imaging in depression. Nucl Med Biol 27:499–507
Gillin JC, Jernajczyk W, Valladares-Neto DC, Golshan S, Lardon M, Stahl SM (1994) Inhibition of REM sleep by ipsapirone, a 5HT1A agonist, in normal volunteers. Psychopharmacology 116:433–436
Goodwin GM, de Souza RJ, Green AR (1985) Presynaptic serotonin receptor-mediated response in mice attenuated by antidepressant drugs and electroconvulsive shock. Nature 317:531–533
Hennig J, Opper C, Huwe S, Netter P (1997) The antagonism of ipsapirone induced biobehavioral responses by ± pindolol in high and low impulsives. J Neural Transm 104:1027–1035
Herrmann WM, Fichte K, Kubicki SK (1980) Definition of EEG frequency bands on the interpretation of factor analysis with EEG power spectrum parameters. In: Kubicki S, Herrmann WM, Laudahn G (eds) Faktorenanalyse und variablenbildung aus dem elektroenzephalogramm. Fischer, Stuttgart, pp 61–74
Howell DC (1999) Fundamental statistics for the behavioural sciences, 4th edn. Duxbury Press, Pacific Grove, Calif.
Koyama T, Meltzer HY (1986) A biochemical and neuroendocrine study of the serotonergic system in depression. In: Hippius H, Klerman GL, Matussek N (eds) New results in depression. Springer, Berlin, pp 169–188
Lerer B, Gelfin Y, Gorfine M, Allolio B, Lesch KP, Newman ME (1999) 5-HT1A receptor function in normal subjects on clinical doses of fluoxetine: blunted temperature and hormone responses to ipsapirone challenge. Neuropsychopharmacology 20:628–639
Maj J, Moryl E (1992) Effects of sertraline and citalopram given repeatedly on the responsiveness of 5-HT receptor subpopulations. J Neural Transm [Gen Sect] 88:143–156
Martin KF, Phillips I, Hearson M, Prow MR, Heal DJ (1992) Characterization of 8-OH-DPAT-induced hypothermia in mice as a 5-HT1A autoreceptor response and its evaluation as a model to selectively identify antidepressants. Br J Pharmacol 107:15–21
Maskall DD, Zis AP, Lam RW, Clark CM, Kuan AJ (1995) Prolactin response to buspirone challenge in the presence of dopaminergic blockade. Biol Psychiatry 38:235–239
McAllister-Williams RH, Young AH (1998) The pathology of depression. A synthesis of the role of serotonin and corticosteroids. In: Ebert D, Ebmeier K (eds) New models for depression. Karger, Basel, pp 170–198
McAllister-Williams RH, Ashton CH, Ferrier IN (1997) Buspirone affects the EEG power spectrum in healthy volunteers: a 5-HT1A effect? J Psychopharmacol 11:A34–133
McCormick DA, Wang Z (1991) Serotonin and noradrenaline excite GABAergic neurones of the guinea-pig and cat nucleus reticularis thalami. J Physiol 442:235–255
Meltzer HY, Maes M (1995) Effects of ipsapirone on plasma cortisol and body temperature in major depression. Biol Psychiatry 38:450–457
Meltzer HY, Maes M (1996) Effect of pindolol on hormone secretion and body temperature: partial agonist effects. J Neural Transm 103:77–88
Meltzer HY, Flemming R, Robertson A (1983) The effect of buspirone on prolactin and growth hormone secretion in man. Arch Gen Psychiatry 40:1099–1102
Mizuki Y, Suetsugi M, Ushijima I, Yamada M (1994) Characteristics of the anxiolytic effects of buspirone in high- and low-anxious normal humans assessed by frontal midline theta activity. Meth Find Exp Clin Pharmacol 16:291–300
Pasqualetti M, Nardi I, Ladinsky H, Marazziti D, Cassano GB (1996) Comparative anatomical distribution of serotonin 1A, 1D alpha and 2A receptor mRNAs in human brain postmortem. Mol Brain Res 39:223–233
Pitchot W, Ansseau M, Gonzalez MA, Lembreghts M, Hansenne M, Wauthy J, Reel C, Jammaer R, Papart P, Sulon J (1995) The flesinoxan 5-HT1A receptor challenge in major depression and suicidal behavior. Pharmacopsychiatry 28:91–92
Power AC, Cowen PJ (1992) Neuroendocrine challenge tests: assessment of 5-HT function in anxiety and depression. Mol Aspects Med 13:205–220
Price LH, Charney DS, Delgado PL, Heninger GR (1991) Serotonin function and depression: neuroendocrine and mood responses

to intravenous l-tryptophan in depressed patients and healthy comparison subjects. Am J Psychiatry 148:1518–1525
Sargent PA, Kjaer KH, Bench CJ, Rabiner EA, Messa C, Meyer J, Gunn RN, Grasby PM, Cowen PJ (2000) Brain serotonin1A receptor binding measured by positron emission tomography with [11C]WAY-100635: effects of depression and antidepressant treatment. Arch Gen Psychiatry 57:174–180
Seifritz E, Moore P, Trachsel L, Bhatti T, Stahl SM, Gillin JC (1996) The 5-HT1A agonist ipsapirone enhances EEG slow wave activity in human sleep and produces a power spectrum similar to 5-HT2 blockade. Neurosci Lett 209:41–44
Seifritz E, Gillin JC, Rapaport MH, Kelsoe JR, Bhatti T, Stahl SM (1998) Sleep electroencephalographic response to muscarinic and serotonin1A receptor probes in patients with major depression and in normal controls. Biol Psychiatry 44:21–33
Seletti B, Benkelfat C, Blier P, Annable L, Gilbert F, de Montigny C (1995) Serotonin1A receptor activation by flesinoxan in humans. Body temperature and neuroendocrine responses. Neuropsychopharmacology 13:93–104
Semlitsch HV, Anderer P, Schuster P, Presslich O (1986) A solution for reliable and valid reduction of ocular artifacts, applied to the P300 ERP. Psychophysiology 23:695–703
Shapira B, Newman ME, Gelfin Y, Lerer B (2000) Blunted temperature and cortisol responses to ipsapirone in major depression: lack of enhancement by electroconvulsive therapy. Psychoneuroendocrinology 25:421–438
Sharp T, McQuade R, Bramwell S, Hjorth S (1993) Effect of acute and repeated administration of 5-HT1A receptor agonists on 5-HT release in rat brain in vivo. Arch Pharmacol 348:339–346
Smith CE, Ware CJ, Cowen PJ (1991) Pindolol decreases prolactin and growth hormone responses to intravenous l-tryptophan. Psychopharmacology 103:140–142
Tunnicliff G (1991) Molecular basis of buspirone's anxiolytic action. Pharmacol Toxicol 69:149–156
Upadhyaya AK, Pennell I, Cowen PJ, Deakin JF (1991) Blunted growth hormone and prolactin responses to l-tryptophan in depression; a state-dependent abnormality. J Affect Disord 21:213–218
Young AH, Sharpley AL, Campling GM, Hockney RA, Cowen PJ (1994) Effects of hydrocortisone on brain 5-HT function and sleep. J Affect Disord 32:139–146