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Serotonin function in anxiety

II. Effects of the serotonin agonist MCPP in panic disorder patients and healthy subjects

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Abstract. To assess the role of serotonin function in the development of panic anxiety, the behavioral and biochemical responses to the serotonin receptor agonist, m-chlorophenylpiperazine (MCPP) was examined in healthy subjects and agoraphobic and panic disorder patients. MCPP had anxiogenic effects in both the healthy subjects and patients. Panic attacks meeting DSM-III criteria occurred following MCPP in 12 of 23 patients and 6 of 19 healthy subjects (NS) and other ratings of anxiety also did not distinguish the two groups. MCPP resulted in significant but similar increases in cortisol, prolactin, and growth hormone in the healthy subjects and patients. The results of this investigation suggest that serotonin neuronal dysfunction may not be of etiologic significance in most panic disorder patients. However, the observed anxiogenic properties of MCPP suggest that additional studies of the role of serotonin systems in the pathophysiology of human anxiety disorders are indicated.

Key words: Serotonin – MCPP – Cortisol – Prolactin – Growth hormone – Anxiety

An involvement of serotonergic neuronal systems in the etiology of anxiety disorders and the mechanism of action of antianxiety drugs has been suggested by preclinical investigations (Geller and Blum 1970; Wise et al. 1972; Engel et al. 1984; Traber et al. 1984). Some animal models of anxiety quantitate the degree to which certain animal behaviors are suppressed by punishment; these behaviors are generally increased following the administration of antianxiety agents such as benzodiazepines (Howard and Pollard 1977). These behaviors can also be increased with chemical depletion of serotonin, surgical lesions of serotonin pathways and serotonin receptor blockade (Geller and Blum 1970; Wise et al. 1972; Stein et al. 1977; Tye et al. 1979; Thiebot et al. 1982; Brady and Barrett 1985a, b). Conversely, increasing serotonin transmission using a variety of electrophysiological and biochemical techniques potentiates the behavioral depressant effects of punishment (Wise et al. 1972; Stein et al. 1973, 1977; Schoenfeld 1976; Graeff and Silveira Filho 1978; Giambalvo and Snodgrass 1978; Simon and Soubrie 1979). Benzodiazepine drugs antagonize these latter effects and it has been suggested that the ability of benzodiazepine drugs (Geller and Blum 1970; Wise et al. 1972; Stein et al. 1977; Nakamura and Fukushima 1977; Tye et al. 1979; Thiebot et al. 1982; Soubrie et al. 1983), as well as some recently developed novel or putative anxiolytic drugs, such as buspirone and TVXQ 7821, (Vander-Maelen and Wilderman 1984; Engel et al. 1984; Traber et al. 1984; Glaser and Traber 1985), to decrease serotonin function is related to their antianxiety properties. Neuroanatomical studies have demonstrated that serotonin innervation of the cerebral cortex is dense and widespread and neurophysiological investigations indicate that serotonin neurons may serve an important function in the integration of sensory information (Steinbusch 1981; Reisine et al. 1982; Pazos and Palacios 1984; Pazos et al. 1985).

Considered together, these studies indicate that increased activity of ascending serotonin pathways may play a role in regulating certain forms of anxiety in animals. A recent preliminary clinical study suggesting that the serotonin receptor antagonist ritanserin may have anxiolytic properties supports this view (Ceulemans et al. 1985). Clinical reports indicating antipanic or antianxiety efficacy for chlorimipramine, a potent serotonin reuptake inhibitor, and the serotonin precursor L-5-hydroxytryptophan also support hypotheses relating serotonin dysfunction to some forms of human anxiety (Gloger et al. 1981; Kahn and Westenberg 1985). However, despite these observations, there have been few clinical investigations designed to evaluate serotonin function in patients with anxiety disorders.

The purpose of the present investigation was to compare serotonin function in panic disorder patients and healthy subjects by determining the behavioral and biochemical responses to the selective serotonin receptor agonist, *m*-chlorophenylpiperazine (MCPP). There is evidence that the ability of MCPP to alter behavior and increase prolactin, cortisol, and growth hormone levels is due to mechanisms involving the stimulation of postsynaptic serotonin receptors, and therefore changes following MCPP administration may serve as a useful index of increased serotonin function (Aloi et al. 1984; Mueller et al. 1985).

Subjects and procedures

Patients. Following an initial screening interview, 28 patients were given a structured interview by a research psychiatrist using the Schedule for Affective Disorders and Schizophrenia (SADS). Five patients did not enter the study because of lack of diagnostic clarity or the presence of a medical problem. Of the final sample of 23 patients who

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completed the study 20 met DSM-III criteria for agoraphobia with panic attacks and three met the criteria for panic disorder. Five of these patients concurrently met DSM-III criteria for major depressive episode without melancholia and two patients met the criteria for major depressive episode with melancholia. In none of these patients did a major depressive disorder precede the onset of their anxiety disorder. The mean age of the 17 female patients was 36 ± 8 years and of the six male patients, 34 ± 7 years. All patients were drug free for at least 3 weeks prior to the initial testing.

The severity of clinical symptoms was assessed weekly by an experienced and trained nurse clinician using the Hamilton Anxiety Scale (HAS) (14 items rated from 0, not present, to 4, very severe; total score from 0 to 56) (Hamilton 1969), Hamilton Depression Scale (HDS) (24 items, rated 0, not present, to 4, severe; total score, 0 to 96) (Hamilton 1960), Clinician Rated Anxiety Scale (CRAS) (36 items rated from 0, not at all, to 4, extremely; total score from 0 to 144), Clinician Global Impression Scale (CGI) (1 item overall life impairment rated from 0, not at all, to 10, very severely) (Zitrin et al. 1983), and a Panic Attack Inventory that documented the type of stimulus and the frequency and severities of panic attacks that occurred during the preceding seven day period. The mean total score ± SD of the available weekly ratings obtained during the 3 weeks surrounding the two MCPP test sessions were calculated for the entire patient group and were as follows: HAS 19 ± 8, HDS 24 ± 11, CRAS 33 ± 13, CGI 5.5 ± 1.4, 5.8 ± 9.8 panic attacks per week.

Healthy subjects. Nineteen healthy subjects were determined to be free of mental disorder on a basis of a structured psychiatric interview and none of the subjects reported a history of mental illness in their first degree relatives. None of the healthy subjects reported taking any psychoactive medication for the 4 weeks prior to the study. Thirteen of the healthy subjects were females (mean age 37 ± 7) and six healthy subjects were males (mean age 38 ± 11).

None of the patients and healthy subjects in the study reported a history of serious medical disease and they all had normal results on physical examination and laboratory testing.

m-chlorophenylpiperazine (MCPP) challenge procedure. Each patient and healthy subject participated in two test sessions. They received an intravenous infusion of placebo (0.45% saline) over 20 min during one test session, and an intravenous infusion of MCPP (0.1 mg/kg) on another test session. The interval between test days was usually 7 days and the interval was not different between the patients and healthy subjects. The 0.1 mg/kg dose of MCPP was chosen because a dose-response study conducted in healthy subjects revealed this dose to produce reliable behavioral and neuroendocrine effects (G.R. Heninger, D.S. Charney, submitted for publication). The order of the placebo and MCPP test sessions was random. Twelve patients and nine healthy subjects received placebo on the first test session, and 12 patients and 10 healthy subjects received MCPP on the first test session.

For each test day the patients fasted overnight for 10 h and remained in the fasting state during the test session. Blood samples for growth hormone, prolactin and cortisol blood samples were drawn at 15 and 0.5 min prior to the MCPP infusion and at 30, 45, 60, 90, 120 and 180 min after the start of the infusion. Sitting and standing blood pressure and pulse rate were obtained at 15 and 0.5 min before and at 30, 60, 90, 120 and 180 min after the start of the MCPP infusion.

Behavioral ratings were administered at 15 and 0.5 min before and at 30, 45, 60, 90, 120 and 180 min after the start of the infusion. Visual analog scales completed by the patients and healthy subjects were used to evaluate the change in ten different mood states (happy, sad, drowsy, anxious, nervous, energetic, calm, fearful, high, mellow). The scales were scored in millimeters from the left hand side of a 100 mm line to a perpendicular mark made by the subjects to the point corresponding to its feeling state at that time. Therefore, the score could range from 0, not at all, to 100, most ever.

The patients and healthy subjects also completed a Panic Attack Symptom Scale (PASS) designed to measure the severity of somatic symptoms commonly associated with states of increased anxiety. Each of the following symptoms were rated on a 4-point scale (1 = not at all to 4 = severe): nausea, urinary frequency, perspiration, palpitations, restlessness, tremors, piloerection, hot and cold flashes, lacrimation, rhinorrhea, muscle aches, numbness, chest discomfort, choking feeling, dizziness, faintness, weakness, feelings of unreality, shortness of breath, fear of death, fear of loss of control, and fear of going crazy.

All patients were assessed as to whether they had had a panic attack during each test session by a research psychiatrist blind to medication states (placebo versus MCPP). Two criteria had to be satisfied: 1) The patient or healthy subject was required to report a crescendo increase in severe subjective anxiety, as reflected by at least a 25 mm rise of the analog rating of anxious, which was accompanied by an increase over baseline on four or more of the 12 DSM-III symptoms of a panic attack, and 2) the patient reported that the anxiety state experienced was similar to that of a naturally occurring panic attack. A healthy subject was considered to have a panic attack if the DSM-III criteria were satisfied.

In addition to the research psychiatrist, the patients, healthy subjects, and research nurses were blind to the sequence of the placebo and MCPP doses.

Biochemical methods. Plasma prolactin was assayed using a radioimmunoassay kit from Sereno Diagnostics, Inc. Intra- and interassay coefficients of variance for this kit were 3 and 7%, respectively. Plasma cortisol was measured using a radioimmunoassay kit supplied by Clinical Assays, Inc. The intra- and interassay coefficients of variance were 3 and 5%, respectively. Plasma growth hormone was measured by a double antibody radioimmunoassay using material supplied by NIAAMD. The intra- and interassay coefficients of variance were 5 and 7%, respectively. To reduce the variance in method, plasma specimens were assayed in duplicate. The individual values reported are the means of two values obtained from the specimens. The laboratory staff were blind to the sequence of the placebo and MCPP doses.

Preparation of MCPP. The MCPP was obtained from the Bristol Myers Company, Pharmaceutical Research and Development Division (Wallingford, CT). A 10 mg sample of the dry material was weighed to within 0.1 mg and diluted

in 10 ml normal saline. Under sterile conditions the solution was passed through a 0.22 micrometer polymer filter. A portion was obtained for pyrogen and sterility testing and the remainder frozen at −20° C until the day of testing. Infusions were carried out utilizing a Harvard Apparatus infusion pump.

Data analysis. The effects of MCPP on behavioral ratings and cardiovascular parameters in the patients and healthy subjects were initially evaluated using an analysis of variance (ANOVA) with repeated measures. This allowed an assessment of the statistical significance of the main effects of group (patients versus healthy subjects), drug (placebo versus MCPP), and time of measurement (changes over the time points sampled). The data analysis was focussed on whether the total patient group reacted differently to MCPP than healthy subjects. In the ANOVA this was manifested in the interaction of group with drug, group with time, and, most important, group with drug and time. The one remaining interaction of drug and time simply indicated the significance of the MCPP effect for the group as a whole. To determine whether different behavioral, biochemical, and cardiovascular effects of MCPP were seen in patients who reported panic attacks, the ANOVA with repeated measures was calculated comparing patients who reported panic attacks to healthy subjects and patients who did not. The significant interactions revealed by ANOVA were further examined with paired and non-paired *t*-tests to assess when significant MCPP effects occurred and whether the patients differed from the healthy subjects in their response to MCPP. This was primarily done by subtracting the baseline value from the value at each time point on the variable of interest. This resulted in a change score at each time point following placebo or MCPP. By subtracting the change following placebo from the change following MCPP, an estimate of the net MCPP effect could be obtained. This is termed the MCPP-placebo difference. The peak change for each variable following MCPP was also measured by subtracting the baseline score from the time point at which the maximal change occurred following MCPP administration. The peak change value following MCPP, minus the peak change following placebo, gave a net peak change of the MCPP effect.

Because of non-normal distributions of the data, non-parametric statistics were used to examine the maximal and temporal effects of MCPP on cortisol, prolactin and growth hormone levels. The Wilcoxon signed rank test was used for within-subject comparisons and between-subject comparisons to evaluate possible healthy subject-patient differences. The baseline prolactin, growth hormone and cortisol values were the mean of the two time points prior to MCPP or placebo because they did not significantly differ.

Pearson and Spearman's rank order correlations were calculated to evaluate the relationship among the biochemical, behavioral and cardiovascular effects of MCPP as reflected by the net peak change in the healthy subjects and patients and the initial clinical ratings of severity in the patients.

The patients with and without concurrent major depression were compared using the types of analysis mentioned above. No significant differences were identified between these groups. Therefore, the results reported represent the entire patient sample.

All results are reported as significant when $P<0.05$ with a two-tailed test. To indicate variance, standard deviations were used.

Results

Behavioral effects of MCPP

Healthy subjects. MCPP had anxiogenic effects in the healthy subjects. Six of the 19 healthy subjects met criteria for experiencing a panic attack following MCPP. None of the healthy subjects experienced a panic attack following placebo. Significant drug and time and sampling interactions were obtained for eight of the ten items rated using the visual analog scales. MCPP caused the patients to rate themselves less happy ($F=5.0$, $df=6,108$, $P<0.001$), less calm ($F=3.8$, $df=6,108$, $P<0.01$), less energetic ($F=5.1$, $df=6,108$, $P<0.001$), more nervous ($F=7.0$, $df=6,108$, $P<0.001$), more anxious ($F=9.1$, $df=6,108$, $P<0.001$), more fearful ($F=2.3$, $df=6,108$, $P<0.05$), more drowsy ($F=3.7$, $df=6,108$, $P<0.01$), and more high ($F=6.5$, $df=6,108$, $P<0.001$).

Peak effects of MCPP on each of the visual analog scale ratings occurred at 30 min. Significant MCPP-placebo changes from baseline in the ratings were as follows: happy, 30 and 60 min; drowsy, 30, 60, 90 and 150 min; calm, 30 min; nervous, 30, 60 and 90 min; anxious, 30, 60, 90 and 120 min; energetic, 30 and 60 min; and high, 30, 60, 90 and 120 min. These significant changes in the visual analog scale ratings were not simply due to changes following placebo because such changes were small and significant alterations following MCPP were similar to those calculated for the MCPP-placebo differences. The mean baseline and MCPP-placebo differences in the ratings of the ten visual analog scale ratings at 30 min are listed in Table 1.

Patients. MCPP has similar effects on the visual analog scale ratings of the patients. Compared to the healthy subjects 12 of the 23 patients met criteria for experiencing a panic attack following MCPP and not following placebo, (NS, patients versus healthy subjects, Fisher exact test). One patient had a panic attack following placebo, but not MCPP. Seven significant drug and time of sampling interactions were found. MCPP caused the patients to rate themselves less happy ($F=6.0$, $df=6,114$, $P<0.001$), less calm ($F=6.5$, $df=6,120$, $P<0.001$), more nervous ($F=7.5$, $df=6,114$, $P<0.001$), more anxious ($F=7.2$, $df=6,120$, $P<0.001$), more fearful ($F=3.4$, $df=6,120$, $P<0.01$), more sad ($F=3.3$, $df=6,120$, $P<0.01$) and more high ($F=2.8$, $df=6,108$, $P<0.05$).

Peak effects of MCPP on each of the visual analog scale ratings also occurred at 30 min. There were significant MCPP-placebo changes from baseline in happy, drowsy, calm, sad, anxious and high at 30 and 60 min, in nervous and fearful at 30 min, drowsy at 90 min, and mellow at 150 min. As with the healthy subjects, the significant MCPP-placebo changes in the visual analog scale ratings could not be substantially accounted for by changes following placebo. The alterations in ratings following placebo were small at 30 min when maximal MCPP effects occurred, except for anxious and nervous for which significant decreases fo 12 mm ($P<0.01$) and 9 mm ($P<0.05$), respectively, were observed at 30 min following placebo. Significant increases from baseline following MCPP were identified for anxious, nervous, and sad at 30 min, drowsy at 30, 60 and

Table 1. The effect of MCPP on self-rated visual analog ratings[a] of ten different mood states in healthy subjects and agoraphobic and panic disorder patients

Ratings	Healthy subjects ($N=19$)		Patients ($N=23$)	
	Baseline	MCPP-placebo change from baseline at 30 min[b]	Baseline	MCPP-placebo change from baseline at 30 min[b]
Happy	52±10	−13±18[e]	24±17[c]	−20±26[e]
Sad	3± 8	1± 8	19±22[c]	15±29[d]
Drowsy	18±18	26±40[e]	22±15	19±41[d]
Calm	51±13	−16±23[e]	30±18[c]	−27±32[d]
Anxious	2± 3	21±22[e]	44±21[c]	34±32[f]
Nervous	1± 2	20±28[e]	40±20[c]	34±34[f]
Fearful	1± 2	7±16	32±25[c]	22±36[e]
Energetic	38±15	−20±28[e,g]	20±15[c]	− 3±19
Mellow	27±21	−10±34	14±18	−10±27
High	6±16	24±32[e]	4± 9	14±25[d]

[a] For each mood state the visual analog scale was a 100 mm line which was rated from 0, not at all, to 100, most ever
[b] Baseline was the mean (±SD) of the two ratings obtained prior to the placebo and MCPP test days. The MCPP-placebo change at 30 min (±SD) refers to the (ratings at 30 min following MCPP minus baseline) minus (rating at 30 min following placebo minus baseline)
[c] $P<0.001$, Baseline, patients vs healthy subjects
[d] $P<0.05$, Change from baseline at 30 min, MCPP vs placebo
[e] $P<0.01$, Change from baseline at 30 min, MCPP vs placebo
[f] $P<0.001$, Change from baseline at 30 min, MCPP vs placebo
[g] $P<0.05$, MCPP-placebo change at 30 min, patients vs healthy subjects

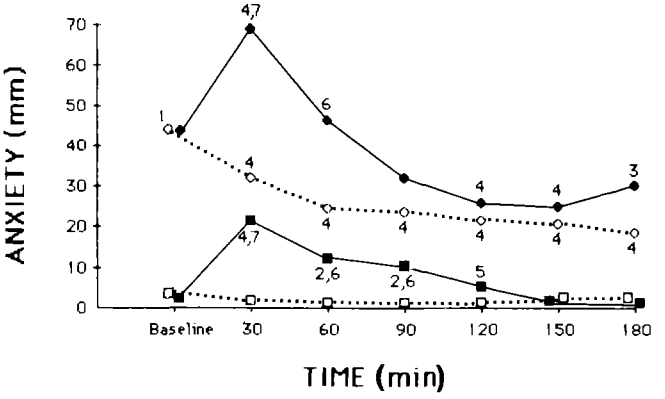

Fig. 1. The ability of MCPP (0.1 mg/kg) to produce anxiety in healthy subjects and agoraphobic and panic disorder patients, as reflected by increases in the self-rated visual analogue scale rating of anxious (100 mm line, rated from 0, not at all, to 100, most ever). At no time point was the MCPP-placebo increase from baseline significantly different between the healthy subjects and agoraphobic and panic disorder patients. *1* $P<0.001$, Baseline, healthy subjects vs patients, student *t*-test (two tailed). *2* $P<0.05$, Time point vs baseline, paired *t*-test (two tailed). *3* $P<0.01$, Time point vs baseline, paired *t*-test (two tailed). *4* $P<0.001$, Time point vs baseline, paired *t*-test (two tailed). *5* $P<0.05$, Change from baseline, MCPP vs placebo, paired *t*-test (two tailed). *6* $P<0.01$, Change from baseline, MCPP vs placebo, paired *t*-test (two tailed). *7* $P<0.001$, Change from baseline, MCPP vs placebo, paired *t*-test (two tailed). □---□ healthy subjects – placebo; ■——■ healthy subjects – MCPP; ○---○ panic disorder patients – placebo; ◆——◆ panic disorder patients – MCPP.

90 min, and high at 30 and 60 min. Ratings of happy and calm were significantly decreased from baseline following MCPP at 30 min. The mean baseline and MCPP-placebo changes from baseline at 30 min for each of the visual analog scale ratings are listed in Table 1.

The ANOVA comparing the 12 patients who did experience panic attacks to the 11 patients who did not revealed no significant group and drug and time interactions for any of the visual analog scale ratings. In addition, at no time point was the MCPP-placebo change from baseline significantly different between these two groups. Baseline visual analog scale ratings were also not significantly different, except that the patients reporting MCPP-induced panic attacks had higher ratings of sad (28±20 versus 10±12, $P<0.05$).

The patients who did and did not experience panic attacks had scores on the HAS, HDS, CRAS, CGI, and panic attack frequency that were similar and not significantly different.

Patient – healthy subject comparison. The patients had significantly higher baseline levels of nervous, sad, anxious, fearful, and lower ratings for happy, calm and energetic (see Table 1). In the comparison of the visual analog scales ratings of the patients and healthy subjects, the three-way interaction of diagnosis and drug and time in the ANOVAs was not significant for any of the items. In addition, at no time point were the MCPP-placebo differences from baseline in any of the behavioral ratings significantly different between the patients and healthy subjects, except for energetic at 30 min (see Table 1). Figure 1 illustrates the temporal effects of MCPP on anxious rating in the healthy subjects and patients.

The ANOVA comparing the 12 patients who did experience MCPP-induced panic attacks to the 13 healthy subjects who did not revealed significant group and drug and time interactions for the visual analog scale ratings of nervous ($F=4.9$, $df=6,120$, $P<0.001$), anxious ($F=2.2$, $df=6,126$, $P<0.05$), fearful ($F=2.6$, $df=6,126$, $P<0.05$), sad ($F=2.2$, $df=6,126$, $P<0.05$) and energetic ($F=3.3$, $df=6,120$, $P<$

Table 2. Significant effects of MCPP on PASS[a] symptoms in healthy subjects and panic disorder patients

PASS symptom	Healthy subjects ($N=19$)		Patients ($N=23$)	
	Baseline	MCPP-placebo change from baseline at 30 min[b]	Baseline	MCPP-placebo change from baseline at 30 min
Nausea	1.0 ± 0	0.8 ± 0.7^{h}	1.3 ± 0.4^{d}	0.8 ± 1.0^{h}
Perspiration	1.0 ± 0	0.3 ± 0.5^{f}	1.2 ± 0.3^{c}	0.5 ± 0.9
Piloerection	1.1 ± 0.2	0.6 ± 0.7^{g}	1.2 ± 0.3	0.5 ± 1.1
Hot & cold flashes	1.0 ± 0.2	0.9 ± 0.9^{h}	1.2 ± 0.3	1.0 ± 1.2^{g}
Lacrimation	1.0 ± 0	0.4 ± 0.5^{g}	1.1 ± 0.3	0.5 ± 1.0^{f}
Rhinorrhea	1.1 ± 0.2	0.1 ± 0.2	1.2 ± 0.3	0.3 ± 0.9
Faintness	1.0 ± 0	1.2 ± 0.8^{h}	1.4 ± 0.5^{d}	1.4 ± 0.9^{h}
Weakness	1.0 ± 0.1	0.7 ± 0.8^{g}	1.5 ± 0.5^{e}	1.1 ± 1.0^{h}
Tremors	1.0 ± 0	0.6 ± 0.8^{f}	1.4 ± 0.5^{e}	$1.2\pm1.1^{h,i}$
Restlessness	1.1 ± 0.2	0.5 ± 1.1	1.6 ± 0.5^{e}	0.5 ± 0.9^{g}
Vertigo	1.0 ± 0	1.1 ± 0.9^{h}	1.1 ± 0.2^{c}	1.0 ± 1.0^{h}
Parathesias	1.0 ± 0	0.6 ± 1.0^{f}	1.2 ± 0.3^{d}	1.0 ± 1.0^{h}
Unreality	1.0 ± 0.1	0.5 ± 0.6^{g}	1.3 ± 0.6	0.9 ± 1.0^{h}
Loss of control	1.0 ± 0	0.3 ± 0.8	1.6 ± 0.7^{e}	0.7 ± 0.9^{h}
Fear of going crazy	1.0 ± 0	$0\ \pm0$	1.3 ± 0.6	0.5 ± 1.2

[a] Each symptom in the 22-symptom Panic Attack Symptom Scale (PASS) was rated on a 4-point scale (1 = not at all to 4 = severe). The symptoms in which a significant drug and time interaction on the ANOVA was identified are listed above (see Methods)

[b] Baseline was the mean ($\pm$SD) of the two ratings obtained prior to the placebo and MCPP test days. The MCPP-placebo change at 30 min ($\pm$SD) refers to the (ratings at 30 min minus baseline following MCPP) minus (ratings at 30 min minus baseline following placebo)

[c] $P<0.05$, Baseline, patients vs healthy subjects, student t-test (two tailed)

[d] $P<0.01$, Baseline, patients vs healthy subjects, student t-test (two tailed)

[e] $P<0.001$, Baseline, patients vs healthy subjects, student t-test (two tailed)

[f] $P<0.05$, Change from baseline, MCPP vs placebo, paired t-test (two tailed)

[g] $P<0.01$, Change from baseline, MCPP vs placebo, paired t-test (two tailed)

[h] $P<0.001$, Change from baseline, MCPP vs placebo, paired t-test (two tailed)

[i] $P<0.1$, MCPP-placebo change from baseline, patients vs healthy subjects, student t-test (two-tailed)

0.01). The patients had a significantly greater MCPP-placebo increase from baseline for nervous (40 ± 26 versus 4 ± 8, $P<0.01$) and anxious (29 ± 21 versus 5 ± 7, $P<0.05$) at 30 min, and sad (18 ± 25 versus -2 ± 7, $P<0.05$) at 60 min after the dose. The healthy subjects had a significantly greater MCPP-placebo decrease from baseline for energetic (2 ± 20 versus -26 ± 26, $P<0.01$) at 30 min after the dose.

Effects of MCPP on somatic symptoms

Healthy subjects. The ANOVA assessing changes in somatic symptoms as measured by the PASS revealed significant drug and time interactions for nausea ($F=15.7$, $df=6,108$, $P<0.001$), numbness ($F=5.2$, $df=6,102$, $P<0.001$), piloerection ($F=10.6$, $df=6,108$, $P<0.001$), hot and cold flashes ($F=9.3$, $df=6,108$, $P<0.001$), lacrimation ($F=4.0$, $df=6,108$, $P<0.001$), dizziness ($F=15.4$, $df=6,102$, $P<0.001$), faintness ($F=27.5$, $df=6,102$, $P<0.001$), tremors ($F=6.3$, $df=6,108$, $P<0.001$), perspiration ($F=2.3$, $df=6,108$, $P<0.05$), anorexia ($F=4.2$, $df=6,102$, $P<0.001$), weakness ($F=8.3$, $df=6,102$, $P<0.001$), feelings of unreality ($F=7.7$, $df=6,102$, $P<0.001$) and feelings of loss of control ($F=2.3$, $df=6,102$, $P<0.05$).

Peak effects of MCPP on somatic symptoms generally occurred at 30 min. MCPP-placebo increases from baseline were significant at one or more time points for each of the variables for which significant interactions were seen. The MCPP-placebo increases from baseline were due to the effects of MCPP because significant effects were not observed following placebo. Table 2 lists the baseline and the MCPP-placebo increases from baseline at 30 min for these variables.

Patients. In the patients, significant drug and time interactions were observed for nausea ($F=6.0$, $df=6,120$, $P<0.001$), perspiration ($F=4.6$, $df=6,120$, $P<0.001$), numbness ($F=7.0$, $df=6,120$, $P<0.001$), restlessness ($F=5.8$, $df=6,120$, $P<0.001$), dizziness ($F=12.2$, $df=6,120$, $P<0.001$), tremors ($F=13.5$, $df=6,120$, $P<0.001$), piloerection ($F=2.4$, $df=6,120$, $P<0.05$), hot and cold flashes ($F=6.5$, $df=6,114$, $P<0.001$), lacrimation ($F=2.9$, $df=6,120$, $P<0.01$), rhinorrhea ($F=2.6$, $df=6,120$, $P<0.05$), feelings of unreality ($F=8.6$, $df=6,120$, $P<0.001$), feelings of weakness ($F=6.5$, $df=6,114$, $P<0.001$), feelings of loss of control ($F=4.4$, $df=6,120$, $P<0.001$) and fear of going crazy ($F=2.8$, $df=6,120$, $P<0.01$). MCPP-placebo increases from baseline were significant at one or more time points for each of the variables for which significant interactions were seen. As with the healthy subjects, the MCPP-placebo increases were due to the effects of MCPP because significant effects were not observed following placebo. Table 2 lists the MCPP-placebo increases from baseline at 30 min for these variables.

The ANOVA comparing the 12 patients who did experience panic attacks to the 11 patients who did not revealed no significant group and drug and time interactions for any of the PASS symptoms. The MCPP-placebo increases from baseline was greater in the patients reporting MCPP-induced panic attacks for almost all of the PASS symptoms,

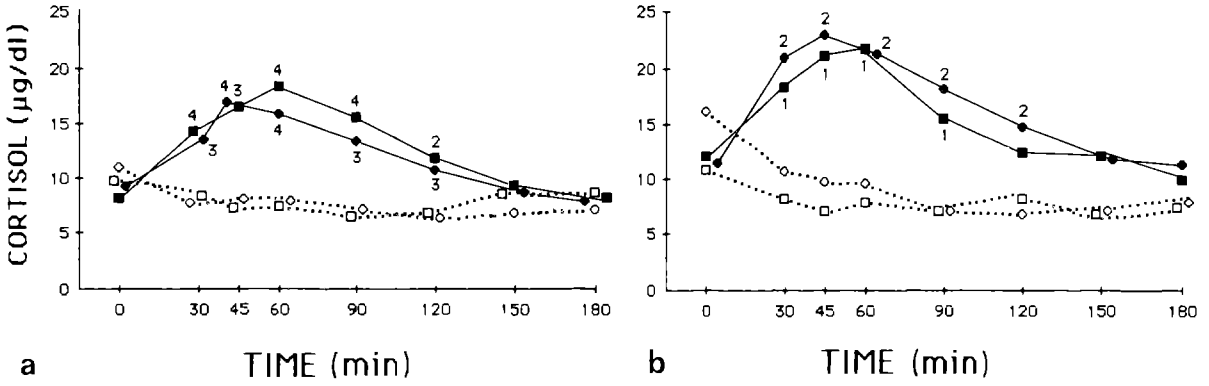

Fig. 2a, b. The ability of MCPP (0.1 mg/kg) to raise cortisol in male ($N=6$) and female ($N=13$) healthy subjects and male ($N=6$) and female ($N=17$) agoraphobic and panic disorder patients. At no time point was the MCPP-placebo increase from baseline significantly different between between healthy subjects and patients. *1* $P<0.1$, Change from baseline, MCPP vs placebo, Wilcoxon signed rank test (two-tailed). *2* $P<0.05$, Change from baseline, MCPP vs placebo, Wilcoxon signed rank test (two-tailed). *3* $P<0.01$, Change from baseline, MCPP vs placebo, Wilcoxon signed rank test (two-tailed). *4* $P<0.001$, Change from baseline, MCPP vs placebo, Wilcoxon signed rank test (two-tailed)
a females; **b** males; □--□ healthy subjects – placebo; ■——■ healthy subjects – MCPP; ◇---◇ panic disorder patients – placebo; ◆——◆ panic disorder patients – MCPP

with significant changes observed for tremors (0.7 ± 1.0 versus -0.0 ± 0.7, $P<0.05$) at 120 min, and feelings of unreality (0.5 ± 0.7 versus 0 ± 0, $P<0.05$) at 180 min. The patients reporting MCPP-induced panic attacks had higher baseline ratings of perspiration, choking feeling, dizziness, faintness, hot and cold flashes, feelings of unreality and loss of control than patients who did not report panic attacks.

Patient – healthy subject comparison. The patients at baseline had higher ratings of nausea, urinary frequency, perspiration, palpitations, numbness, restlessness, chest pain, choking feeling, vertigo, faintness, tremors, lacrimation, weakness, shortness of breath, fear of death and loss of control. In the comparison of the healthy subjects and patients, the ANOVA revealed significant diagnosis and drug and time interactions only for tremors ($F=2.6$, $df=6,228$, $P<0.05$). The MCPP-placebo increases in tremors from baseline were significantly greater in the patients at 60 min (0.3 ± 0.6 versus 0.9 ± 1.1, $P<0.05$). There was a trend for a greater increase in tremors in the patients at 30 min (Table 2).

The ANOVA comparing the 12 patients who reported MCPP-induced panic attacks to the 13 healthy subjects who did not revealed significant group and drug and time interactions for palpitations, tremors, faintness, feeling of unreality and feelings of loss of control. Significantly greater MCPP-placebo increases from baseline were found for palpitations at 60 (0.6 ± 0.6 versus 0.1 ± 0.3, $P<0.05$) and 180 min (0.5 ± 0.7 versus 0 ± 0, $P<0.01$); faintness at 120 (0.7 ± 0.9 versus 0 ± 0, $P<0.01$) and 150 min (0.5 ± 0.7 versus 0 ± 0, $P<0.01$); tremors at 30 (1.6 ± 1.0 versus 0.5 ± 0.8, $P<0.01$), 60 (1.2 ± 1.0 versus 0.2 ± 0.4, $P<0.01$) and 120 (0.7 ± 1.0 versus 0 ± 0, $P<0.05$) min; feelings of unreality at 180 min (0.5 ± 0.2 versus 0 ± 0, $P<0.05$); and feelings of loss of control at 30 min (0.9 ± 0.7 versus 0.1 ± 0.3, $P<0.01$). The patients who experienced MCPP-induced panic attacks had higher baseline ratings of perspiration, choking feeling, dizziness, faintness, hot and cold flashes, feelings of unreality and loss of control than the healthy subjects who did not report panic attacks. There was no correlation between baseline ratings and the behavioral changes induced by MCPP in either the patients or healthy subjects.

Neuroendocrine effects of MCPP

Cortisol. Following placebo, there were significant decreases from baseline in both groups ranging from 1.8 to 5.7 µg/dl at all time points except at 120, 150 and 180 min in the healthy subjects. Significant increases from baseline ranging from 4.6 to 9.9 µg/dl following MCPP were observed in the healthy subjects and patients at 30, 45, 60 and 90 min. Significant MCPP-placebo increases from baseline were observed in both groups at 30, 45, 60, 90 and 120 min and in the patients only at 150 min (Fig. 2).

Prolactin. Following placebo there were significant decreases ranging from -0.6 to -0.9 ng/ml from baseline at 30, 45 and 60 min in both groups. Significant increases from baseline ranging from 2.6 to 7.5 ng/ml following MCPP were observed in the healthy subjects and patients at every time point. Significant MCPP-placebo increases were observed in both groups at every time point (Fig. 3).

Growth hormone. Following placebo there were no significant changes from baseline in either group. Significant increases from baseline ranging from 2.2 to 4.5 ng/ml after MCPP were observed in the healthy subjects and patients at 90 and 120 min and at 60 min in the patients only. Significant MCPP-placebo increases from baseline were observed at 90, 120 and 150 min in the healthy subjects and patients and at 45 min in the patients only (Fig. 4).

Patient – healthy subject comparisons. There were no significant differences in baseline levels of cortisol, prolactin and growth hormone between the healthy subjects and patients. The comparison of the cortisol, prolactin and growth hormone responses to MCPP in the healthy subjects and patients revealed that at no time point was there a significant difference in the MCPP-placebo increases in these hor-

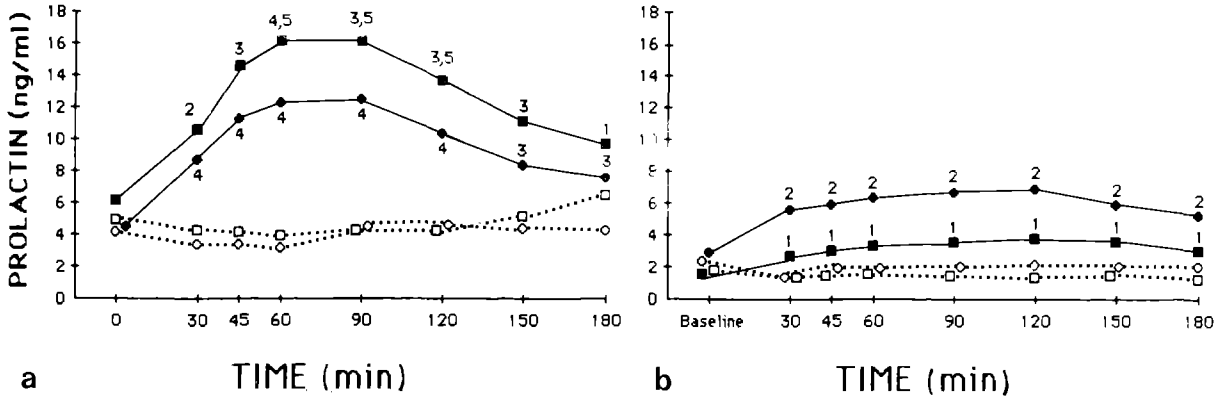

Fig. 3a, b. The ability of MCPP (0.1 mg/kg) to raise prolactin in male and female healthy subjects and agoraphobic and panic disorder patients. At no time point was the MCPP-placebo increase from baseline significantly different between healthy subjects and patients. *1* $P<0.1$, Change from baseline, MCPP vs placebo, Wilcoxon signed rank test (two-tailed). *2* $P<0.05$, Change from baseline, MCPP vs placebo, Wilcoxon signed rank test (two-tailed). *3* $P<0.01$, Change from baseline, MCPP vs placebo, Wilcoxon signed rank test (two-tailed). *4* $P<0.001$, Change from baseline, MCPP vs placebo, Wilcoxon signed rank test (two-tailed). *5* $P<0.05$, MCPP-placebo increase from baseline, female healthy subjects vs male healthy subjects, Wilcoxon signed rank test (two-tailed)
a females; **b** males; □---□ healthy subjects – placebo; ■——■ healthy subjects – MCPP; ◇---◇panic disorder patients – placebo; ◆——◆ panic disorder patients – MCPP

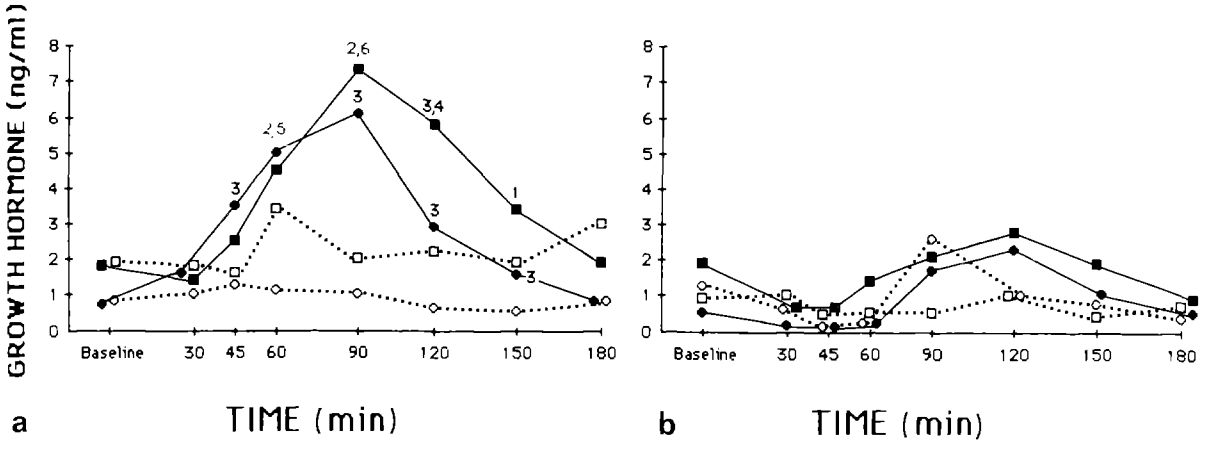

Fig. 4a, b. The ability of MCPP (0.1 mg/kg) to raise growth hormone in male and female healthy subjects and agoraphobic and panic disorder patients. At no time point was the MCPP-placebo increase from baseline significantly different between healthy subjects and patients. *1* $P<0.1$, Change from baseline, MCPP vs placebo, Wilxocon signed rank test (two-tailed). *2* $P<0.05$, Change from baseline, MCPP vs placebo, Wilcoxon signed rank test (two-tailed). *3* $P<0.01$, Change from baseline, MCPP vs placebo, Wilcoxon signed rank test (two-tailed). *4* $P<0.001$, Change from baseline, MCPP vs placebo, Wilcoxon signed rank test (two-tailed). *5* $P<0.1$, MCPP-placebo increase from baseline, female patients vs male healthy subjects, Wilcoxon signed rank test (two-tailed). *6* $P<0.05$, MCPP-placebo increase from baseline, female healthy subjects vs male healthy subjects, Wilcoxon signed rank test (two-tailed)
a females; **b** males; □---□ healthy subjects – placebo; ■——■ healthy subjects – MCPP; ◇---◇ panic disorder patients – placebo; ◆——◆ panic disorder patients – MCPP

mones from baseline between the two groups (see Figs. 2, 3 and 4).

The patients who reported MCPP-induced panic attacks did not have significantly different baseline levels or greater increases in cortisol, prolactin or growth hormone after MCPP in comparison to the healthy subjects and to the patients who did not have panic attacks. Significant MCPP-placebo differences in cortisol, growth hormone or prolactin secretion were not observed at any time points between these groups.

There was no correlation between baseline hormone levels and MCPP-induced increases in these hormones. The only significant correlation between MCPP-induced changes in behavior and hormone secretion was a correlation net peak increase in cortisol and the net peak increase in anxious ratings ($r=0.61$, $P<0.01$) in the patients. A similar significant correlation was not observed in the healthy subjects ($r=0.03$, $P=0.91$).

Cardiovascular effects of MCPP

Healthy subjects. In the healthy subjects, the ANOVA revealed significant drug and time interactions for sitting systolic ($F=4.6$, $df=6,108$, $P<0.001$) and diastolic blood pressure ($F=2.5$, $df=6,108$, $P<0.05$) and sitting heart rate ($F=2.3$, $df=6,108$, $P<0.05$). Significant MCPP-placebo increases from baseline were found for sitting systolic blood

pressure at 30 min (8 ± 3, $P < 0.05$) and sitting heart rate at 60 (8 ± 2, $P < 0.01$) and 90 (6 ± 3, $P < 0.05$) min.

Patients. In the patients, significant drug and time interactions were not identified for blood pressure or heart rate. Significant MCPP-placebo increases from baseline were observed for sitting diastolic blood pressure at 30 (5 ± 2, $P < 0.05$) and 60 (3 ± 2, $P < 0.05$) min and sitting heart rate at 120 (6 ± 2, $P < 0.05$) min.

The ANOVA comparing the MCPP-induced changes in blood pressure and heart rate in the patients who experienced MCPP-induced panic attacks to patients who did not revealed no significant group and drug and time interactions for blood pressure or heart rate. A significantly greater MCPP-placebo increase from baseline in sitting systolic blood pressure at 180 min (6 ± 3 versus -7 ± 4, $P < 0.05$) was seen in patients reporting panic attacks compared to the other patients.

Patient – healthy subject comparison. There were no baseline differences in blood pressure or heart rate between the healthy subjects and the patients. The ANOVA comparing the healthy subjects and patients revealed no significant group and drug and time interactions for blood pressure or heart rate and at no time point were MCPP-placebo changes from baseline significantly different between the two groups. Similar results were obtained when patients who reported MCPP induced panic attacks where compared to healthy subjects who did not. The only baseline differences between these groups was that patients reporting panic attacks had significantly higher baseline standing heart rate (84 ± 7 versus 76 ± 9, $P < 0.05$) compared to patients who did not and significantly higher sitting (78 ± 6 versus 70 ± 9, $P < 0.05$) and standing (84 ± 7 versus 75 ± 10, $P < 0.05$) heart rate compared to healthy subjects who did not report panic attacks.

There was no correlation between baseline blood pressure or heart rate and MCPP-induced changes in these variables. In addition, there was no correlation between MCPP-induced changes in blood pressure or heart rate and the behavioral and neuroendocrine effects of MCPP.

Relationship of MCPP effects to gender

The effects of MCPP on the visual analog ratings of mood state, the PASS, blood pressure and heart rate were similar in male and female healthy subjects and patients as reflected by nonsignificant interactions between gender (male versus female), drug and time of sampling and nonsignificant *t*-tests at the time points following drug administration.

The ability of MCPP to increase prolactin and growth hormone appeared to be greater in the female healthy subjects and patients. Within-group comparisons indicated that the MCPP-placebo increases from baseline in prolactin and growth hormone were greater in magnitude and more highly significant in the female healthy subjects and patients compared to the male healthy subjects and patients. Between-group comparisons indicated that the MCPP-placebo increase from baseline in growth hormone tended to be significantly greater at 60 min in the female patients compared to the male patients. The MCPP-placebo increase from baseline in growth hormone was significantly greater at 90 min and in prolactin at 60, 90 and 120 min in the female healthy subjects compared to male healthy subjects. Baseline prolactin levels were significantly higher in the female healthy subjects (5.5 ± 2.3 versus 1.6 ± 1.0, $P < 0.001$) and female patients (4.9 ± 4.3 versus 2.3 ± 0.5, $P < 0.05$) compared to male healthy subjects and patients. Baseline growth hormone and cortisol levels were not different between male and female healthy subjects and patients.

The findings regarding the comparative behavioral, biochemical and cardiovascular responses to MCPP in the healthy subjects and patients were not altered when the analysis was done comparing male healthy subjects to male patients and female healthy subjects to female patients. Figures 2, 3 and 4 illustrate the effects of placebo and MCPP on prolactin, cortisol and growth hormone levels in female and male healthy subjects and patients.

Correlations with clinical severity

The behavioral, neuroendocrine and cardiovascular responses to MCPP did not appear to be specifically related to clinical severity of illness. The only significant correlations were found between the ratings of clinical severity and significant responses to MCPP was that the Hamilton Anxiety Scale scores correlated significantly with peak changes in vertigo ($r = 0.44$, $P < 0.05$), tremors ($r = 0.42$, $P < 0.05$), lacrimation ($r = 0.54$, $P < 0.01$) and rhinorrhea ($r = 0.41$, $P < 0.05$).

Discussion

The finding of the present investigation that panic disorder patients exhibit similar anxiogenic responses and increases in prolactin, cortisol and growth hormone following MCPP compared to healthy subjects suggests that serotonin function may not be primarily involved in the etiology of panic anxiety disorders. The interpretation and evaluation of the data are largely dependent on the validity of using the behavioral responses and increases in prolactin, cortisol and growth hormone following MCPP as a means to assess serotonin function.

Validity of MCPP infusion as an index of serotonin function. MCPP is a metabolite of the novel antidepressant, trazodone, and appears to have selective effects on serotonin function (Caccia et al. 1982). MCPP has been shown to readily penetrate the central nervous system, with higher concentrations of MCPP achieved in brain than in plasma following systemic administration (Caccia et al. 1981; Fuller et al. 1981). In laboratory animals MCPP, in doses of 2.5 mg/kg or lower, produces a decrease in brain levels of the serotonin metabolite, 5-hydroxyindoleacetic acid, but does not alter brain levels of the dopamine metabolite, homovanillic acid, or the norepinephrine metabolite, 3-methoxy-4-hydroxyphenylethyleneglycol (Fuller et al. 1981; Invernizzi et al. 1981). MCPP has been shown to potently inhibit the uptake of serotonin. Receptor binding studies indicate that MCPP has strong affinity for $5HT_1$ receptors, although controversy exists regarding the selectivity of MCPP for $5HT_{1A}$ and $5HT_{1B}$ sites (Saminin et al. 1979; Fuller et al. 1981; Invernizzi et al. 1981; Sills et al. 1984; Peroutka 1985). MCPP does not potently displace 3H-WB4101, an alpha-1 adrenergic receptor ligand, 3H-dihydroalprenolol, a beta-adrenergic receptor ligand, or 3H-spiroperiodol from rat striatal membranes which labels dopamine receptor binding sites (Invernizzi et al. 1981). Taken

together, these preclinical studies suggest that MCPP in low doses may be a selective serotonin receptor agonist. It is probably via this action that MCPP increases prolactin, cortisol and growth hormone and produces behavioral changes.

The results from a large number of animal studies on serotonergic control of prolactin release indicates that serotonin increases prolactin secretion by suprahypophyseal mechanisms, probably involving the mediobasal hypothalamus (Birge et al. 1970; Kamberi et al. 1971; Kordon et al. 1973; Mueller et al. 1976; Clemens et al. 1978; Lamberts and MacLeod 1978; Garthwaite and Hagen 1979). Electrical or pharmacological activation of the dorsal raphe nucleus, a major source of serotonin neurons in the hypothalamus, increase serum prolactin whereas serotonergic stimulation of prolactin release is antagonized by dorsal raphe and mediobasal hypothalamus lesions (Meites et al. 1977; Advis et al. 1979; Van de Kar and Bethea 1982).

The effect of serotonin on cortisol secretion appears to be mediated by a different mechanism than the effect of serotonin on prolactin levels (Van de Kar et al. 1985). It has been demonstrated that the serotonergic stimulation of cortisol secretion is not mediated by either the dorsal or median raphe nucleus (Van de Kar et al. 1982, 1985). There is evidence that brain serotonin neurons can stimulate the release of corticotropin releasing factor into hypophyseal portal blood, which results in enhanced ACTH release from the anterior pituitary and cortisol output from the adrenal gland (Gibbs and Vale 1983). There is also evidence that serotonin can affect ACTH release by acting directly at the anterior pituitary level (Fuller and Snoddy 1980; Fuller 1981) and increase cortisol output at the level of the adrenal cortex (Gibbs and Vale 1983).

The role of serotonin neurons in the stimulation of growth hormone release has not been established, with both stimulatory and inhibitory effects reported (Weiner and Ganong 1978; Meites and Sonntag 1981). The site at which serotonin might act to increase growth hormone secretion also requires clarification because of conflicting data whether pituitary serotonin receptors exist which enhance growth hormone secretion when stimulated (Dorsa and Conners 1979).

The greater neuroendocrine responses to MCPP in females are consistent with preclinical investigations demonstrating increased serotonin function in female rats (Carlsson et al. 1985). These studies indicate that serotonergic neurons of the female rat brain have a greater storage capacity and a higher enzymatic activity with a higher rate of serotonin synthesis (Carlsson et al. 1985). It has been suggested that this sex difference in the functional activity of the serotonin system may be due to enhancement of serotonin function by ovarian steriods (Munaro 1978) or inhibition of serotonin function by androgens (Fischette et al. 1984). These observations are also consistent with the finding that the prolactin rise following tryptophan is greater in female healthy subjects compared to male healthy subjects and female depressed patients compared to male depressed patients (Heninger et al. 1984).

Serotonin function and panic anxiety. The normal prolactin, cortisol and growth hormone responses to MCPP in panic disorder patients are consistent with the results of a previous investigation that the prolactin response to tryptophan is also normal in panic disorder patients (Charney and Heninger 1986). The normal neuroendocrine responses to MCPP in panic disorder patients are also consistent with the results of two of three investigations that platelet imipramine binding is normal in panic disorder patients (Lewis and McChesney 1985; Uhde et al. 1985; Innis et al. 1987). Radiolabeled imipramine has been shown to label a saturable, high affinity binding site in brain and platelet. Several lines of evidence suggest that this binding site is associated with, although not identical to the serotonin reuptake site. These results in panic disorder patients differ from the findings in depressed patients of a blunted prolactin response to tryptophan and reduced platelet imipramine binding (Langer et al. 1980; Paul et al. 1980; Heninger et al. 1984).

The demonstration that MCPP produces anxiety symptoms in both the healthy subjects and patients supports a link between some forms of anxiety and increased postsynaptic serotonin activity. This finding may also be viewed as consistent with the hypothesis that an ability to reduce presynaptic serotonin neuronal activity by activation of inhibitory 5HT-$_{1A}$ somatodendritic autoreceptors is related to anxiolytic properties (Glaser and Traber 1983, 1985; Vander Maelen and Wilderman 1984; Engel et al. 1984). In contrast to the robust inhibitory effects of potent 5HT-$_{1A}$ agonists, MCPP has weak or irregular actions on the spontaneous activity of 5HT neurons (Sprouse and Aghajanian 1987).

The finding that MCPP in 32% and 52% of the healthy subjects and patients, respectively, produced a combination of anxiety and somatic symptoms meeting DSM-III criteria for a panic attack suggests that some forms of panic anxiety may be related to abnormalities in serotonergic function. The lack of significant difference in the frequency of MCPP-induced panic attacks between the healthy subjects and patients may be due to a number of factors, including: (1) the sample size studied may have been too small to reveal significant differences and (2) the dose of MCPP used may have been too high to determine if patients and healthy subjects had a different threshold for MCPP-induced anxiety.

Several types of investigations designed to further study the role of serotonin in the pathophysiology of anxiety appear to be logical next steps. It will be important to assess the effects of lower doses of MCPP in panic disorder patients and healthy subjects. The measurement of presynaptic serotonin neuronal activity as well as the function of other serotonin receptor subtypes will yield critical data regarding whether net serotonin activity is increased in some panic disorder patients. Drug efficacy studies in panic disorder patients involving medications with specific and potent effects on serotonin function will help ascertain whether an ability to enhance serotonin activity is related to antipanic activity.

The observation that MCPP can induce panic anxiety, considered with the results of previous investigations documenting the panicogenic properties of caffeine and yohimbine (Charney et al. 1984, 1985; Uhde et al. 1984, 1985), indicates that drugs which produce similar panic anxiety states can have markedly different actions on neurotransmitter function, hormone secretion, and cardiovascular function. An implication of these findings is that current diagnostic criteria for panic attacks probably define a behavioral state that is neurobiologically heterogenous. This emphasizes the limitations of the use of descriptive methods for psychiatric classification designed for etiological pur-

poses and the need for clinically applicable biological tests capable of determining specific neurobiological dysfunctions in psychiatric disorders.

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