Saccadic peak velocity and EEG as end-points for a serotonergic challenge test

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We previously reported that a single dose of the serotonin receptor agonist meta-chlorophenylpiperazine increased the peak velocity of saccadic eye movements and decreased low-frequency electroencephalographic activity.

Methods We administered a single dose of the serotonin releaser dexfenfluramine in a double blind, placebo controlled randomised cross-over design and measured saccadic eye movements and EEG every hour up to 6 h. Subjects were 62 males (18–30 years) with a history of no, moderate or heavy use of ecstasy tablets.

Results Dexfenfluramine increased saccadic peak velocity and decreased alpha, delta and theta electroencephalographic activity, the latter predominantly in heavy users of ecstasy.

Conclusions This study supports the idea that saccadic peak velocity and EEG can be useful endpoints of a serotonergic challenge. This could be an important anatomical extension of these end-points, which until now were limited to the effect on hypothalamic serotonergic projections. Copyright © 2002 John Wiley & Sons, Ltd.

Key words — pharmacokinetics; pharmacodynamics; EEG; saccadic eye movements; serotonin; dexfenfluramine; PKPD; ecstasy; MDMA

INTRODUCTION

We reported earlier that a single dose of meta-chlorophenylpiperazine (mCPP) increased the peak velocity of saccadic eye movements and decreased electroencephalographic occipital theta activity, both in a concentration dependent way (Gijsman et al., 1998). Single dose administration of serotonergic agents, such as mCPP and dexfenfluramine, are well-established challenge tests to evaluate the functional status of the serotonergic system of the brain (Cowen et al., 1990). The usual pharmacodynamic endpoint of these tests is the increase of plasma levels of cortisol, prolactin and other neurohormones (Tuomisto and Mannisto, 1985). This effect is probably mediated through serotonergic neurons in the raphe nuclei in the pons and upper brainstem that project to the hypothalamus (Van de Kar, 1991). The increase of peak velocity is indicative of arousal, but it is an uncommon finding because this measure is more sensitive to decrease. Decreases of saccadic peak velocity are routinely used to quantify the effect of sedative drugs, such as benzodiazepines (Bahill et al., 1981; Van Steveninck et al., 1994). In this study we report the effect of single dose dexfenfluramine on saccadic eye movements and electroencephalography as a further exploration of the value of these parameters in serotonergic challenge tests. Our hypothesis was that dexfenfluramine would increase saccadic peak velocity and decrease slow wave electroencephalographic activity. We also hypothesised that these effects would be hampered by the extent of previous use of ‘ecstasy’. These data were collected as part of a study in visitors of rave-parties with different levels of drug use.

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of previous use of ‘ecstasy’ tablets. We reported elsewhere that the cortisol response to dexfenfluramine was negatively related to the frequency of ecstasy use during the previous 2 years (Verkes et al., 2001).

### METHODS

#### Participants

The Medical Ethics Review Board of Leiden University Medical Centre approved the study and all participants gave written informed consent. We recruited 62 regular visitors of rave parties: 20 non-users who had never used ecstasy (mean age 20.6 year (SD 2.2 year), weight 73.8 kg (SD 11.1 kg)), 21 moderate users who had used ecstasy on 12 to 48 separate occasions during the past 2 years (mean age 22.1 (2.3), weight 74.9 (8.4)), and 21 heavy users with use on more than 48 occasions (mean age 21.7 (2.8), weight 71.5 (10.7)). A history of alcohol- or substance-dependence or a major psychiatric disorder during the last year led to exclusion. Participants were instructed to maintain a regular lifestyle and not to use any psychotropic agents, including all illegal drugs from 1 week before the first test occasion until the last test occasion. This was checked by urine tests at all visits. We described extensive details of the recruitment procedure and the participants elsewhere (Verkes et al., 2001).

#### Dexfenfluramine challenge test

We performed a double blind, crossover challenge-test of 30 mg dexfenfluramine or placebo. There was a washout-period of at least 5 days.

#### Laboratory analyses

For all assays blood was drawn at 0, 1, 2, 3, 4, 5 and 7 h. For the assay of dexfenfluramine and its active metabolite nordexfenfluramine 9 ml whole blood was drawn in a Greiner Vacuette plain tube, tilted and stored at 4°C. Within 1 h after collection it was centrifuged at 4°C for 10 min at 1500 g. Plasma was stored directly at −20°C. We measured the plasma concentrations of dexfenfluramine and nordexfenfluramine by gas chromatography with mass selective detection after derivatisation with trifluoroacetic anhydride. The lower limit of quantification was 1 ng/ml and the assay linearity was from 1 ng/ml to at least 100 ng/ml. Cortisol and prolactin samples were prepared and measured as described previously (Gijsman et al., 1998).

#### Electro-oculography

Disposable AgCl electrodes were fixed to the temples, after skin resistance was reduced to less than 5 k Ohm. A fixed support for the head restrained any movement. During the study days, saccadic eye movements were recorded at 0, 1, 2, 3, 4, 5 and 6 h. Each recording session consisted of 15 saccades of 15 degrees total angle. Data were analysed using a microcomputer-based system for sampling and analysis of eye movements (Cambridge Electronics Design Ltd. Cambridge, England). Saccadic peak velocity, saccadic latency and accuracy were evaluated (Bahill et al., 1981; Van Steveninck et al., 1994). Participants performed three recording sessions at the screening day to eliminate the adaptation effect.

#### Electroencephalography

EEG was registered at 0, 1, 2, 3, 4, 5 and 6 h at leads Fz-Cz and Pz-Oz as described elsewhere (Gijsman et al., 1998). EEG recordings were made using silver-silver chloride electrodes, fixed with collodion at Fz, Cz, Pz and Oz, with the same common ground electrode as for the eye movement registration (international 10/20 system). The electrode resistances were kept below 5 k Ohm. All recordings were done with the subjects’ eyes closed. EEG signals were obtained from leads Fz-Cz and Pz-Oz. The signals were amplified by use of a Nihon Kohden AB-621G bioelectric amplifier (Nihon Kohden Corporation, Tokyo, Japan) with a time constant of 0.3 s and a low pass filter at 100 Hz. analogue/digital conversion was performed with a CED1401 intelligent interface (Cambridge Electronics Design Ltd. Cambridge, England), using a sampling rate of 1024 Hz. Per session eight consecutive blocks of 8 s were recorded over a 2 min period. Datablocks containing artefacts were identified by visual inspection and these were excluded from analysis. The frequency bandwidth was 50 Hz. Fast Fourier transform analysis was performed to obtain the sum of amplitudes in the delta- (0.5–3.5 Hz), theta- (3.5–7.5 Hz), alpha- (7.5–11.5 Hz) and beta- (11.5–30 Hz) frequency ranges.

#### Statistical analysis

We estimated the pharmacokinetics of dexfenfluramine and nordexfenfluramine using the following equation:

\[
C_t = \frac{C_{\text{max}} (t-\text{lag})}{T_{\text{max}}} e^{-(t-\text{lag})/T_{\text{max}}}
\]

where \( t \) is time, \( T_{\text{max}} \) is the time of \( C_{\text{max}} \) (maximum concentration), and \( \text{lag} \) is the lag-time. This is the
equation describing first-order oral absorption and single-compartment elimination where an absorption half-life and elimination half-life cannot be distinguished. Parameters were estimated using non-linear mixed effect modelling as implemented in NONMEM V (NONMEM Project Group, University of California, San Francisco, California, USA).

Pharmacodynamic effects were calculated as differences from placebo of averages over time. Participants were excluded from analysis of a pharmacodynamic parameter when any time-point was missing. Response to dexfenfluramine was compared between users groups using one-way ANOVA. Differences were reported with 95% confidence intervals (95% CI). We performed these calculations using SPSS for Windows V6.1.2 (SPSS, Inc., Chicago, Illinois, USA).

Population estimates were calculated to predict concentrations at the times of pharmacodynamic assessments. From this we derived individual empirical Bayes estimates, which we inspected visually. NONMEM was used to estimate concentration-effect relationships with the actual pharmacodynamic measurements and the predicted concentrations.

RESULTS

Missing data

Data from all EEG and eye movement recordings were missing for one participant from the moderate users group, as a result of technical problems. Data for posterior/occipital EEG for another five participants (3 from the non-users group and 2 from the moderate users group) were incomplete and so excluded for analysis.

Pharmacokinetics

Table 1 shows the pharmacokinetic parameters of dexfenfluramine and nordexfenfluramine. The time course of the plasma levels of dexfenfluramine and nordexfenfluramine is depicted in Figure 1.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SEM</th>
<th>CV</th>
<th>Mean ± SEM</th>
<th>CV</th>
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<tbody>
<tr>
<td><strong>Dexfenfluramine</strong></td>
<td></td>
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<tr>
<td>AUC (ng/mL.min)</td>
<td>5556 ± 179.7</td>
<td></td>
<td>2739 ± 95.6</td>
<td></td>
</tr>
<tr>
<td>(C_{max}) (ng/mL)</td>
<td>18.0 ± 0.605</td>
<td>26%</td>
<td>10.4 ± 0.300</td>
<td>21%</td>
</tr>
<tr>
<td>(T_{max}) (min)</td>
<td>196 ± 4.99</td>
<td>17%</td>
<td>414 ± 20.5</td>
<td>26%</td>
</tr>
<tr>
<td>Lag-time (min)</td>
<td>42.1 ± 2.10</td>
<td>24%</td>
<td>39.9 ± 3.29</td>
<td>24%</td>
</tr>
</tbody>
</table>

AUC, area under the curve; \(C_{max}\) is maximum concentration; \(T_{max}\) is time of \(C_{max}\); CV is coefficient of variation.

Figure 1. Average plasma levels and standard deviation of dexfenfluramine (●) and nordexfenfluramine (○) (n = 62)
Pharmacodynamics

The average over time of saccadic peak velocity was higher after dexfenfluramine compared with placebo but did not differ between groups (Table 2, Figure 2). Saccadic accuracy and reaction time did not change.

Posterior/occipital delta and theta activity was lower after dexfenfluramine compared with placebo for the whole group and for the heavy users group. No differences were present between groups (Table 2).

Frontal/central alpha activity was significantly lower in the heavy users group after dexfenfluramine compared with placebo. This effect was significantly different from the moderate users group.

Pharmacokinetic/pharmacodynamic relationships

There was no clear pattern in line-graphs of the PK/PD relationships in individual subjects. There was a significant positive concentration-effect relationship of dexfenfluramine as well as of nordexfenfluramine with saccadic peak velocity (Table 3).

DISCUSSION

This study confirms the possible usefulness of saccadic peak velocity and EEG parameters as an end-point in serotonergic challenge tests, because as hypothesised dexfenfluramine caused a concentration-dependent increase of saccadic peak velocity and a decrease of slow wave EEG activity. In contrast with our hypotheses, the effect on saccadic peak velocity was not modified by previous use of ecstasy. Moreover, the effect on slow wave EEG was greater in heavy users of ecstasy rather than weaker as we expected. Finally, dexfenfluramine also decreased EEG alpha activity compared with placebo in heavy users, an effect which differed from that in moderate users.

The effect on saccadic peak velocity is comparable to the effect of mCPP, a serotonin-2c-receptor-agonist (Gijsman et al., 1998). This finding also supports the theory that the effect is mediated through serotonergic neurons, because dexfenfluramine—other than mCPP—exclusively acts via serotonin (5-HT) receptors. Nordexfenfluramine has a higher affinity for this serotonin-2c-receptor than dexfenfluramine and could therefore be responsible for the effect (Gibson et al., 1993). However, this is unlikely since the peak of the effect precedes the peak of the concentration of nordexfenfluramine by at least 3 h.

<table>
<thead>
<tr>
<th>Table 2: Pharmacodynamics</th>
<th>ANOVA</th>
<th>p</th>
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<tr>
<td><strong>Saccadic eye movements</strong></td>
<td></td>
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<tr>
<td>Peak velocity (mean/SD)</td>
<td>494 (38)</td>
<td>177 (9.1, 26.3)</td>
</tr>
<tr>
<td>Latency (ms)</td>
<td>0.21 (0.021)</td>
<td>0.19 (0.009)</td>
</tr>
<tr>
<td>EEG (μV)</td>
<td>4.0 (1.1)</td>
<td>17.7 (9.1, 26.3)</td>
</tr>
<tr>
<td>FC Alpha</td>
<td>4.2 (0.21)</td>
<td>17.7 (9.1, 26.3)</td>
</tr>
<tr>
<td>PO Alpha</td>
<td>3.1 (0.30)</td>
<td>17.7 (9.1, 26.3)</td>
</tr>
<tr>
<td>PO Delta</td>
<td>3.8 (0.13)</td>
<td>17.7 (9.1, 26.3)</td>
</tr>
<tr>
<td>PO Theta</td>
<td>3.1 (0.30)</td>
<td>17.7 (9.1, 26.3)</td>
</tr>
<tr>
<td>PO Theta</td>
<td>3.1 (0.30)</td>
<td>17.7 (9.1, 26.3)</td>
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Saccadic peak velocity may be a welcome extension to the end-points of the challenge test, which until now were mainly limited to endocrine parameters regulated by the hypothalamus. However, the question remains through which part of the brain this serotonergic effect is mediated (Bergquist et al., 1999).

Areas involved in saccadic eye movements are the frontal cortex and the colliculus superior that send initialising signals to the saccadic generator in the paramedian pontine reticular formation (PPRF) which are adjusted by the cerebellum (Bittencourt and Tedeschi, 1990). The peak saccadic velocity specifically reflects the activity of this generator. The cerebellum seems not involved in our effect, as the accuracy of the saccades remained unchanged. The omnipausal neurons in the PPRF seem less likely to mediate this effect, because they receive only very limited serotonergic input (Bittencourt and Tedeschi, 1990; Horn et al., 1994). The colliculus superior may be less likely to mediate the effect because in animals it is severely affected by the neurotoxic effects of 3,4-methylenedioxyamphetamine (MDA), the most neurotoxic component of ecstasy tablets (Harvey et al., 1993). We would therefore expect a hampered effect related to previous use of ecstasy. An alternative explanation could be that saccadic peak velocity is mediated by large serotonergic axons, which are unaffected by 3,4-methylenedioxyamphetamine (MDMA) the main component of ecstasy tablets. MDMA has been reported to mainly affect fine serotonergic axons (Wilson et al., 1989). However, the neurotoxicity of ecstasy in humans is still a matter of debate and harmful effects in animals may not be similar to those in humans.

In the cortex, dexfenfluramine causes opposite bilateral effects on left and right frontal glucose utilisation (Mann et al., 1996). Additional arguments in favour of this location are that dexfenfluramine increased frontal glucose utilisation (Kapur et al., 1994; Meyer et al., 1996) and saccadic eye movements were associated with increased frontal cerebral blood flow (O’Driscoll et al., 1998). Alternatively, the
orbitofrontal and occipital cortex cannot be completely ruled out as being involved in the effect (Doudet et al., 1995).

The main EEG finding was that dexfenfluramine in the central posterior-occipital region decreased delta and theta activity, while no effect occurred in the fronto-central area. This is in agreement with the findings of others that 30 mg dexfenfluramine decreased slow wave activity all over the brain apart from the central region (Saletu et al., 1993). In our study this effect mainly seems to originate from the group of heavy users of ecstasy, although the other groups show a trend in the same direction and are statistically not different from the heavy users.

The decrease of EEG alpha activity in heavy users is somewhat surprising. This finding seems in disagreement with others who reported a limited increase in alpha activity after 30 mg dexfenfluramine (Saletu et al., 1993). In that study they also reported a dose-related increase of beta activity after dexfenfluramine, but this occurred predominantly in the left anterior cortex which we did not record in our study. The cause of the significant difference between the moderate and heavy users is difficult to explain. The moderate users show a trend to increased alpha activity, making a linear relation of the effect with previous ecstasy exposure unlikely. Possibly this is an effect related to past or recent use of ecstasy that occurs in heavy users only. We reported in the original study that heavy users in comparison with moderate users have significantly lower current alcohol intake (average 0.9 vs 1.4 units per day) and also fewer days since the last ecstasy use before the dexfenfluramine challenge (average 19 days vs 28 days) (Verkes et al., 2001). These factors cannot be excluded from mediating this effect, but it seems unlikely that these quantitatively small differences cause such a specific effect on the EEG. Altogether, heavy previous use of ecstasy seems to strengthen the effect on slow wave EEG parameters, to reverse the expected effect on fast-wave EEG activity and to have no modifying effect at all on the increase in saccadic peak velocity. This would be consistent with a general slowing down of cortical activity, while the apparently more centrally generated eye movements remain unaffected. These findings demand further exploration using full-lead EEG in users of ecstasy and in controls.

In conclusion, saccadic peak velocity and EEG may be useful additional end-points of serotonergic challenge tests. Future studies need to address further characteristics of this parameter, such as generalisability to other serotonergic drugs and dose-response relationships. Its exact mechanism and the location of action requires further investigation. Future studies should combine eye movements with contingent multi-channel EEG and/or other imaging techniques of the brain.

ACKNOWLEDGEMENTS

This study was fully funded by a research grant from the Department of Health, Welfare and Sports of the Netherlands. We wish to acknowledge the expert assistance of the nursing staff of CHDR. Dexfenfluramine was kindly provided by Les Laboratoires Servier, Neuilly-sur-Seine, France.

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