Cyproheptadine prevents pergolide-induced valvulopathy in rats: an echocardiographic and histopathological study

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Droogmans S, Roosens B, Cosyns B, Degaillier C, Hernot S, Weytjens C, Garbar C, Caveliers V, Pipeleers-Marchal M, Franken PR, Lahoutte T, Schoors D, Van Camp G. Cyproheptadine prevents pergolide-induced valvulopathy in rats: an echocardiographic and histopathological study. Am J Physiol Heart Circ Physiol 296: H1940–H1948, 2009. First published April 3, 2009; doi:10.1152/ajpheart.01177.2008.—Serotonergic drugs, such as pergolide, have been associated with the development of cardiac valvular myxoid thickening and regurgitation in humans and more recently in rats. These effects are potentially mediated by the 5-hydroxytryptamine (5-HT2BR) receptor (5-HT2BR). Therefore, we sought to determine whether cyproheptadine, a 5-HT2BR antagonist, might prevent toxic valvulopathy in an animal model of pergolide-induced valvular heart disease. For this purpose, 50 male Wistar rats received daily intraperitoneal injections of pergolide (0.5 mg/kg, n = 14), pergolide (0.5 mg/kg) combined with cyproheptadine (10 mg/kg, n = 12), cyproheptadine (10 mg/kg, n = 12), or no injections (control, n = 12) for 20 wk. Echocardiography was performed blindly at baseline and at 10 and 20 wk followed by pathology. At baseline, no differences between groups were found with echocardiography. At 20 wk, aortic regurgitation was present in all pergolide-treated animals, whereas it was less frequently observed in the other groups (P < 0.0001). For the other valves, this difference was less pronounced. On histopathology, not only aortic but also mitral valves were thicker, myxoid, and exhibited more 5-HT2BR-positive cells in pergolide-treated animals compared with the other groups. Moreover, regurgitant aortic and mitral valves were thicker than nonregurgitant aortic and mitral valves. In conclusion, we found that cyproheptadine prevented pergolide-induced valvulopathy in rats, which was associated with a reduced number of 5-HT2BR-positive valvular cells. This may have important clinical implications for the prevention of serotonergic drug-induced valvular heart disease.

MATERIALS AND METHODS

Study Design

Fifty male Wistar Unilever rats (Harlan, 330 ± 3 g, 9 wk) were divided into four groups. All products were given as daily intraperitoneal injections for 20 wk. The first group (n = 14) received pergolide (0.5 mg/kg), whereas the second group (n = 12) received pergolide (0.5 mg/kg) combined with cyproheptadine (10 mg/kg). In the second group, both injections were administered with an interval of between 15 and 30 min. The third group (n = 12) received cyproheptadine (10 mg/kg), whereas the fourth group (n = 12) of control rats received no injections. Echocardiography was performed before the injections and at 10 and 20 wk followed by necropsy and histological examination of the heart. The study protocol was approved by the Ethics Committee for Animal Studies of Vrije Universiteit Brussel. Guidelines from the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animal Care (NIH Pub. No. 85-23, Revised 1985) were followed.

Animal Handling

During the whole study, animals were housed in stainless steel cages with sawdust bedding. They were kept at an average room temperature of 24°C, a relative humidity of 50%, and a 12:12-h day-night cycle. Food (rat maintenance diet, SAFE) and water were provided at libitum.

SEVERAL DRUGS, such as ergotamine, methysergide, fenfluramine, pergolide, and cabergoline, have been associated with valvular heart disease (1, 22, 26, 31, 34). Hereby, the 5-hydroxytryptamine (5-HT2BR) receptor (5-HT2BR) has been postulated as the key pathway through which these drugs activate valvular fibroblasts to produce myxoid substance and collagen (25, 28). This leads to valvular thickening and stiffening with valvular regurgitation as a consequence.

Pergolide, a dopamine agonist widely used for the treatment of Parkinson’s disease, has recently been voluntarily withdrawn from the United States market because of two publications confirming its toxic valvular effect (26, 34). At this moment, pergolide is still used by neurologists and endocrinologists outside the United States because of its unique therapeutic properties. Switching to other dopamine agonists without or with less 5-HT2BR agonist activity might be difficult in patients who have had sustained benefit and stable motor disorders. The Federal Drug Administration cautions against abruptly stopping pergolide and is looking for ways to provide the drug to those people who cannot successfully be switched to alternative treatments.

We (3) recently developed a small animal model of pergolide-induced valvular heart disease in the rat. This model has the potential to screen high-risk drugs for valvular toxicity and to evaluate treatment strategies.

Cyproheptadine is a cheap, widely available H1 and 5-HT antagonist used for the treatment of allergic reactions and prophylaxis of migraine and the symptomatic treatment of metastatic carcinoid syndrome. It has also pronounced 5-HT2BR antagonistic properties (Ki = 1.4 nM) (33) and was therefore recently proposed by Roth (24) as a theoretical candidate for the prevention of drug-induced valvular heart disease. Therefore, we hypothesized that cyproheptadine coadministration with pergolide could prevent toxic valvulopathy in rats. Such a treatment strategy could have important clinical implications.
Drug Preparation and Administration

Pergolide mesylate (Sigma-Aldrich) was prepared in a 10% ethanolic solution in saline at a concentration of 0.5 mg/ml. Cyproheptadine hydrochloride sesquihydrate (Sigma-Aldrich) was prepared in 25% DMSO (VWR) solution in aqua ad injectabilia at an initial concentration of 40 mg/ml. In the first 2 wk, four animals died of intestinal obstruction in the cyproheptadine-treated groups. This was probably due to the anticholinergic effect at that dosage. Therefore, the concentration was decreased to 10 mg/ml after 2 wk. The treatment identity of the rats was hidden during echocardiography and pathological evaluation.

Echocardiography

Ten minutes before being imaged, the rat was anesthetized with 50 mg/kg pentobarbital sodium (Nembutal, CEVA, Brussels, Belgium) intraperitoneally. Subsequently, the anterior chest wall was shaved, and the rat was placed in left lateral decubitus on a wooden bench to obtain optimal image quality and views, as previously described (3, 32).

A Vivid 7 Pro system (GE Medical Systems, Milwaukee, WI, USA) with a 10-MHz neonatal probe (10S) and 13-MHz linear probe (13L) was used. Detailed acquisition and processing regarding regurgitant jet evaluation and quantification of left ventricular (LV) systolic and diastolic function have been previously reported (4). Briefly, regurgitant jets were assessed visually in all possible views. The color-Doppler ratio of the regurgitant aortic jet width to the LV outflow tract diameter and the mitral and tricuspid valve regurgitant area ratio (jet/atrium) were calculated. The relative duration of the regurgitant jet on color M-mode to diastole (for pulmonary and aortic valves) or systole (for tricuspid and mitral valves) was also determined. LV volumes were calculated from the M-mode tracings in a short-axis view (papillary muscle level, average of 3 cycles) using the following ellipsoid model: \( \pi \times D^3/3 \) (where \( D \) is the diameter of the ventricle in the short-axis view) (23). The LV ejection fraction (LVEF) was determined from the LV end-diastolic volume (LVEDV) and LV end-systolic volume (LVESV) as follows: \( \text{LVEF} = [(\text{LVEDV} - \text{LVESV})/\text{LVEDV}] \times 100. \) Cardiac output was calculated as stroke volume \( \times \) heart rate, with stroke volume equal to LVEDV minus LVESD. The fractional area change (FAC) was calculated by the LV cross-sectional area at end diastole (LVAd) and end systole (LVVs) using the following equation: \( \text{FAC} (\text{in} \%) = [(\text{LVAd} - \text{LVes})/\text{LVad}] \times 100 \) (30). The following LV diastolic parameters were also assessed: peak early velocity, peak late velocity, and mitral medial diastolic annulus velocity.

Influence of Drug Administration on Blood Pressure During Echocardiography

Blood pressure was measured with the advanced autoinflate blood pressure monitor with an infrared sensor and tail cuff from Harvard Apparatus in a subset of 32 rats (8 rats/group). Systolic and diastolic blood pressures were measured after 10 and 20 min after the pentobarbital (50 mg/kg ip) injection. Pentobarbital was administered 30 min after injection of the study drug.

Histopathology

At the end of the experiment, animals were killed with 120 mg/kg pentobarbital sodium intravenously. Hearts were excised, weighed, and fixed in neutral buffered formalin (10%) for 2 h. Hearts were then embedded in paraffin and cut in an axial plane (from base to apex). Three ranges of sections (4–6 μm thickness) were carried out with \( \sim 100 \) μm between each range to visualize the maximum amount of valvular tissue, and slides were stained with hematoxylin, eosin, saffranin (HES), alcian blue, and Masson’s trichrome. Immunohistochemical staining for 5-HT_{3}Rs was performed with rabbit anti-5-HT_{3}R antibody (10 μg/ml, n°ab13292, Abcam, Cambridge, MA) according to the manufacturer’s instructions and as done by Elangbam et al. (8). After incubation with biotinylated donkey anti-rabbit IgG (dilution \( \times 300 \), n° RPN1004, Amersham Biosciences), an alkaline phosphatase detection system (Vectastain ABC-AP standard kit) was used with fucsin + substrate chromogen (Dako) without counterstaining. Morphometry was performed by digital image analysis using a personal computer digital image camera (Digital Sight DS-5M, Nikon) mounted on an Axiolab Zeiss light microscope (Carl Zeiss).

We used the NIH Image program (Image-J 1.35d, NIH, Bethesda, MD). The program was calibrated with a graduated slide. Microscopic images were used to blindly evaluate cardiac valves and cardiomyocytes. The maximum thickness of each valve was measured. The width of at least 10 LV cardiomyocytes was measured in each section. Semi-quantitative compositional analysis of the valves was carried out for glycosaminoglycans (GAGs; alcian blue) and collagen (trichrome). Therefore, microscopic images were taken under the same conditions (magnification: \( \times 100 \)) and were converted to 8-bit images. An appropriate threshold was determined on one slide for GAGs and collagen content, respectively, and subsequently kept constant. Areas of interest were drawn around the valvular leaflets, and the proportion of GAG to collagen was calculated. For immunohistochemistry, the absolute number of 5-HT_{3}R-positive cells in valvular leaflets were counted in microscopic images taken at a magnification of \( \times 200 \).

Statistical Analysis

Data are expressed as means \( \pm SD \). Comparisons between groups were performed using the Mann-Whitney \( U \)-test for continuous variables and Fisher’s exact test for categorical variables. All \( P \) values were calculated two tailed. \( P \) values of \( <0.05 \) were considered significant. Statistical analysis was done with SPSS software (version 16.01, SPSS, Chicago, IL).

RESULTS

General Characteristics

In the pergolide + cyproheptadine-treated group, three animals died (two animals within 2 wk and one animal at week 14), whereas three animals in the cyproheptadine-treated group died (two animals within 2 wk and one animal at week 9). At necropsy, the four rats that died before the first 2 wk showed an intestinal obstruction at the level of the colon due to fecal impaction. In the two other rats, no clear cause of death could be detected. After a reduction to 10 mg/kg, cyproheptadine was well tolerated by the other animals. Animals that received pergolide showed hyperactivity, poor grooming, aggressive behaviour, and increased gnawing activity, which was more pronounced during the first week of the study. This was also seen in the pergolide + cyproheptadine-treated group. On the other hand, the cyproheptadine-treated group showed no general behavioral changes.

Echocardiography

Valves. All valves were visualized with echocardiography during the study. At baseline, there were no differences in valvular regurgitation between groups (data not shown). The occurrence and severity of valvular regurgitation in the different groups are shown in Tables 1 and 2. One rat in the cyproheptadine-treated group at 20 wk was excluded from the echographic analysis because of too-deep sedation leading to bradycardia.

At 20 wk, aortic regurgitation was present in all 14 pergolide-treated rats compared with 1 rat in the pergolide + cyproheptadine-treated group (11%), 2 rats in the cyproheptadine-
treated group (25%), and 1 rat in the control group (8%, \( P < 0.0001 \)). Aortic regurgitation was already more frequent at 10 wk in pergolide-treated rats compared with the other groups.

At 20 wk, mitral regurgitation was found in seven rats in the pergolide-treated group (50%) compared with four rats in the pergolide + cyproheptadine-treated group (44%, \( P = 1.000 \)), none in the cyproheptadine-treated group (0%, \( P = 0.022 \)), and four rats in the control group (33%, \( P = 0.453 \)). Although the mean mitral regurgitant area ratio was not different between groups, the mean relative duration of the mitral regurgitation to systole was higher in the pergolide-treated group compared with the other groups (Table 2).

Tricuspid and pulmonary regurgitation were already found in the control group (39% with regurgitation), and mitral valves were thicker than nonregurgitant aortic valves. Pergolide-treated animals exhibited an increased number of 5-HT2BR-positive cells compared with control animals (597 ± 51 g, \( P = 0.003 \); 519 ± 61 g, \( P = 0.001 \); and 523 ± 51 g, \( P = 0.002 \), respectively) were lower compared with control animals (597 ± 30 g).

Valves. On histopathological examination, pergolide-treated rats had thickened aortic and mitral valves compared with the other groups. Aortic and mitral valve thickening were prevented with cyproheptadine (Fig. 1). The tricuspid valve was not clearly affected by pergolide. In addition, regurgitant aortic and mitral valves were thicker than nonregurgitant aortic (273 ± 176 vs. 180 ± 84 \( \mu \)m, \( P = 0.029 \)) and mitral (419 ± 152 vs. 260 ± 118 \( \mu \)m, \( P = 0.003 \)) valves (Fig. 2).

Pergolide-treated animals exhibited an increased number of 5-HT2BR-positive interstitial and endothelial cells in the aortic and mitral leaflets (Fig. 3) compared with the other groups. Most of the 5-HT2BR-positive cells were scattered throughout the fibromyxoid sponge layer.

### Pathology

**Postmortem body weight.** Body weights of pergolide-, pergolide + cyproheptadine-, and cyproheptadine-treated animals (540 ± 44 g, \( P = 0.003 \); 519 ± 61 g, \( P = 0.001 \); and 523 ± 51 g, \( P = 0.002 \), respectively) were lower compared with control animals (597 ± 30 g).

### Table 1. Occurrence of TR, PR, MR, and AR at 10 and 20 wk in all groups

<table>
<thead>
<tr>
<th></th>
<th>Cyproheptadine</th>
<th>Pergolide + Cyproheptadine</th>
<th>Pergolide</th>
<th>Control, %</th>
<th>%</th>
<th>P value vs. control</th>
<th>%</th>
<th>P value vs. control</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TR</td>
<td>33</td>
<td>33</td>
<td>1.000</td>
<td>30</td>
<td>1.000</td>
<td>71</td>
<td>0.113</td>
<td>0.102</td>
</tr>
<tr>
<td>PR</td>
<td>33</td>
<td>11</td>
<td>0.338</td>
<td>20</td>
<td>0.646</td>
<td>43</td>
<td>0.701</td>
<td>0.176</td>
</tr>
<tr>
<td>MR</td>
<td>25</td>
<td>0</td>
<td>0.229</td>
<td>30</td>
<td>1.000</td>
<td>29</td>
<td>1.000</td>
<td>0.127</td>
</tr>
<tr>
<td>AR</td>
<td>0</td>
<td>0</td>
<td>1.000</td>
<td>0</td>
<td>1.000</td>
<td>36</td>
<td>0.042*</td>
<td>0.116</td>
</tr>
<tr>
<td>20 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TR</td>
<td>42</td>
<td>63</td>
<td>0.650</td>
<td>56</td>
<td>0.670</td>
<td>86</td>
<td>0.038*</td>
<td>0.309</td>
</tr>
<tr>
<td>PR</td>
<td>58</td>
<td>38</td>
<td>0.650</td>
<td>44</td>
<td>0.670</td>
<td>71</td>
<td>0.683</td>
<td>0.187</td>
</tr>
<tr>
<td>MR</td>
<td>33</td>
<td>0</td>
<td>0.117</td>
<td>44</td>
<td>0.673</td>
<td>50</td>
<td>0.453</td>
<td>0.022*</td>
</tr>
<tr>
<td>AR</td>
<td>8</td>
<td>25</td>
<td>0.537</td>
<td>11</td>
<td>1.000</td>
<td>100</td>
<td>&lt;0.0001*</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

Shown are percentage of rats with valvular regurgitation in each group as well as the corresponding \( P \) values. TR, tricuspid regurgitation; PR, pulmonary regurgitation; MR, mitral regurgitation; AR, aortic regurgitation. *Statistically significant difference.

LV function. There were no differences at baseline in LV systolic and diastolic parameters (data not shown). LV functional parameters at 20 wk are shown in Table 3. Heart rate was highest in the cyproheptadine-treated group. LV systolic function (FAC and LVEF) was lowest in the pergolide-treated group. This was less pronounced in the pergolide group. This was less pronounced in the pergolide group. This was less pronounced in the pergolide group.

**Influence of drug administration on blood pressure during echocardiography.** No acute effects on blood pressure were found between pergolide-treated (120 ± 14/80 ± 4 mmHg, \( P = 0.755/5 = 0.573 \)), pergolide + cyproheptadine-treated (141 ± 14/92 ± 9 mmHg, \( P = 0.161/5 = 0.161 \)), cyproheptadine-treated (136 ± 14/88 ± 9 mmHg, \( P = 0.108/5 = 0.108 \)), and control (125 ± 18/81 ± 12 mmHg) rats (mean blood pressure was measured after 10 and 20 min after the pentobarbital injection, given 30 min after the study drug).

**Table 2. Severity of valvular regurgitation in the different treatment groups at 20 wk**

<table>
<thead>
<tr>
<th></th>
<th>Cyproheptadine</th>
<th>Pergolide + Cyproheptadine</th>
<th>Pergolide</th>
<th>Control, %</th>
<th>%</th>
<th>P value vs. control</th>
<th>%</th>
<th>P value vs. control</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR jet width/LVOT</td>
<td>23</td>
<td>29±6</td>
<td>0.667</td>
<td>31</td>
<td>1.000</td>
<td>35±9</td>
<td>0.267</td>
<td>0.417</td>
</tr>
<tr>
<td>Relative duration of AR to diastole, %</td>
<td>17</td>
<td>17±1</td>
<td>1.00</td>
<td>20</td>
<td>1.000</td>
<td>22±8</td>
<td>0.80</td>
<td>0.70</td>
</tr>
<tr>
<td>Mitral regurgitant area ratio</td>
<td>11±3</td>
<td></td>
<td></td>
<td>8±3</td>
<td>0.343</td>
<td>13±8</td>
<td>0.927</td>
<td>0.23</td>
</tr>
<tr>
<td>Relative duration of MR to systole, %</td>
<td>12±3</td>
<td></td>
<td></td>
<td>13±2</td>
<td>0.686</td>
<td>20±8</td>
<td>0.024*</td>
<td>0.024*</td>
</tr>
<tr>
<td>Tricuspid regurgitant area ratio</td>
<td>9±3</td>
<td>9±2</td>
<td>0.841</td>
<td>9±3</td>
<td>0.690</td>
<td>12±5</td>
<td>0.221</td>
<td>0.180</td>
</tr>
<tr>
<td>Relative duration of TR to systole, %</td>
<td>14±3</td>
<td>16±2</td>
<td>0.286</td>
<td>16±4</td>
<td>0.413</td>
<td>15±3</td>
<td>0.339</td>
<td>0.679</td>
</tr>
<tr>
<td>Relative duration of PR to diastole, %</td>
<td>19±1</td>
<td>22±4</td>
<td>0.267</td>
<td>17±2</td>
<td>0.412</td>
<td>18±4</td>
<td>0.475</td>
<td>0.161</td>
</tr>
</tbody>
</table>

LVOT, left ventricular (LV) outflow tract diameter. *Statistically significant difference.
Valvular thickening by pergolide was due to myxoid transformation of the sponge layer with an increase of the GAG-to-collagen ratio (Fig. 4). This was most pronounced in the mitral valve. GAG deposits were also observed in the other groups but were smaller and localized at the distal free edge of the valvular leaflets. In contrast, pergolide-treated rats showed mostly diffuse thick myxoid changes reaching the base of the cusps. Chondroid metaplasia was found in all groups between Table 3.

<table>
<thead>
<tr>
<th></th>
<th>Ciproheptadine</th>
<th>Pergolide + Ciproheptadine</th>
<th>Pergolide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Cyproheptadine</td>
<td>P value vs. control</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>313 ± 21</td>
<td>351 ± 24</td>
<td>0.005*</td>
</tr>
<tr>
<td>Anterior wall at diastole, mm</td>
<td>1.88 ± 0.15</td>
<td>1.93 ± 0.19</td>
<td>0.910</td>
</tr>
<tr>
<td>LV end-diastolic diameter, mm</td>
<td>8.37 ± 0.47</td>
<td>7.76 ± 0.62</td>
<td>0.047*</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>77.3 ± 3.7</td>
<td>77.2 ± 3.9</td>
<td>0.970</td>
</tr>
<tr>
<td>Fractional area change, %</td>
<td>62.3 ± 2.9</td>
<td>63.8 ± 4.4</td>
<td>0.442</td>
</tr>
<tr>
<td>Cardiac output, ml/min</td>
<td>149 ± 25</td>
<td>134 ± 30</td>
<td>0.270</td>
</tr>
<tr>
<td>E/A</td>
<td>1.21 ± 0.07</td>
<td>1.44 ± 0.0</td>
<td>0.400</td>
</tr>
<tr>
<td>E/A'</td>
<td>15.6 ± 1.7</td>
<td>16.1 ± 2.0</td>
<td>0.750</td>
</tr>
</tbody>
</table>

There were 12 rats in the control group, 8 rats in the cyproheptadine-treated group, 9 rats in the pergolide + cyproheptadine-treated group, and 14 rats in the pergolide-treated group. E, peak early velocity; A, peak late velocity; E', mitral medial diastolic annulus velocity. *Statistically significant difference.

Valvular thickening by pergolide was due to myxoid transformation of the sponge layer with an increase of the GAG-to-collagen ratio (Fig. 4). This was most pronounced in the mitral valve. GAG deposits were also observed in the other groups but were smaller and localized at the distal free edge of the valvular leaflets. In contrast, pergolide-treated rats showed mostly diffuse thick myxoid changes reaching the base of the cusps. Chondroid metaplasia was found in all groups between

Fig. 1. Top: photomicrographs of aortic and mitral valves in control, cyproheptadine-treated, pergolide + cyproheptadine-treated, and pergolide-treated animals. Pergolide-treated rats demonstrated diffuse thickened aortic and mitral valves, in contrast to the other groups. Arrows indicate the valvular leaflets. Sections were stained with HES. Magnification: ×25. Scale bar = 300 μm. Bottom: maximum aortic and mitral valve thicknesses in the different groups. Values (in μm) are expressed as means ± SD; n, number of valves analyzed in each group. *P < 0.05; **P < 0.01.
the attachments side of the left coronary aortic cusp and anterior mitral leaflet.

LV myocytes. LV myocytes were thicker in the pergolide-treated group compared with the other groups. LV fibrosis was not found (Fig. 4).

DISCUSSION

In this study, we found that cyproheptadine had a preventive effect against the development of pergolide-induced valvulopathy in a rat model. This was demonstrated by a reduction of incidence of aortic regurgitation on echocardiography and by less myxoid aortic and mitral thickening on histology. This effect was related to a reduction of 5-HT2BR-positive valvular cells. Our data further indicated that echocardiography was a reliable tool to assess the presence of pergolide-induced valvulopathy and follow treatment with cyproheptadine and also correlated well with histopathology.

Cyproheptadine protected against pergolide-induced aortic regurgitation in this study, whereas its effect on the incidence of mitral regurgitation was not significant. This could be due to the relatively lower frequency of mitral regurgitation detected in the pergolide-treated group compared with previous work (50% vs. 67%) (3). On the other hand, the relative duration of mitral regurgitation was more pronounced in pergolide-treated animals compared with the other groups. On histopathology, pergolide + cyproheptadine-treated rats had clearly less affected aortic and mitral valves compared with the pergolide-treated group, which demonstrates the protective effect of cyproheptadine on left-sided heart valves. Moreover, on histology, regurgitant aortic and mitral valves were thicker than nonregurgitant valves. The absence of a significant change in blood pressure between the different groups suggests that the observed valvular regurgitations are not importantly determined by blood pressure and rather due to true valvular lesions.

5-HT2BR activation is a common feature of all valvulopathic drugs (or their metabolites), whereas this was not found with drugs not associated with valvular heart disease (10, 25, 28, 29). Moreover, some of these valvulopathic drugs have been shown to induce mitogenesis in cultured interstitial cells from human cardiac valves by activating the 5-HT2BR (28). In the present study, we found that cyproheptadine reduced the number of 5-HT2BR-positive cells in the aortic and mitral valvular leaflets when given with pergolide, which further suggests the involvement of the 5-HT2BR in the pathogenesis of drug-induced valvulopathy. This is also strengthened by the observation that chemically similar drugs, such as lisuride and terguride, which are agonists for 5-HT2CRs and 5-HT2ARs and antagonists for 5-HT2BRs, were not associated with valvulopathy (15, 24). Similarly, no increase in the risk of valvulopathy was observed in patients treated with the nonergot-derived dopamine agonist pramipexole, which has a low affinity for the 5-HT2BR (26, 34). Moreover, 5-HT injections in rats also led to increased 5-HT2BR mRNA expression in the affected valves (7). In another animal study (13), terguride inhibited the long-term hypertrophic organ effects of 5-HT. Young et al. (33) recently published a full binding profile of cyproheptadine. Besides the 5-HT2BR, cyproheptadine also antagonizes several other 5-HT receptors. Hence, the exact mechanism by which cyproheptadine prevented pergolide valvulopathy in this study needs to be further investigated.

We could not observe a clear toxic effect of pergolide on the tricuspid valve, as it was less affected in histology and echocardiography. Moreover, toxic right-sided valvular heart disease is more difficult to diagnose and evaluate, as functional (physiological) regurgitations are more pronounced in both humans and rats. This is also true for the pulmonary valve. With our technique, this valve was very difficult to visualize on histopathology because of its more anterior-superior position.
As in our previous study (3), no sufficient pulmonary valves could be histologically evaluated to draw meaningful conclusions.

In aging rats, spontaneous nodular and segmental myxoid valvular thickening has been described (5, 6). This was also found in the control rats of this study. However, compared with the pergolide-treated group, valvular thickening was less pronounced and more localized toward the distal free edges of the valvular leaflets. This can as well explain the progressive increase in the number of tricuspid, pulmonary, and mitral regurgitations in control rats during the study. On the other hand, serotonergic compounds such as serotonin and pergolide led to fibroblast stimulation with a more pronounced and diffuse accumulation of GAGs throughout the entire valve. Similar histological changes have also been described in anorexigen-induced valvulopathy and floppy mitral valves in humans, as these valves contained more GAGs than normal (16). Several studies (3, 7, 12) have shown that serotonin injections also led to a similar valvulopathy in rats. A recent study (7) by Elangbam et al. has shown that rats treated with high-dose 5-HT exhibited, after only 7 days, thickened aortic and mitral valves with increased GAG and decreased collagen content. In humans, normal valves are composed of equal amounts of GAG and collagen (17). Similar observations were made in this study, where the thickened valves (especially the mitral valve) of pergolide-treated rats had an increased GAG-to-collagen content, whereas the other groups had an equal distribution of GAG and collagen.

Besides functional and morphological valvular alterations, pergolide also gave rise to myocardial hypertrophy without fibrosis. This was accompanied with a decrease of LV function. Similar findings were observed in our previous study (3). The absence of LV hypertrophy in the pergolide/H11001 cyproheptadine-treated group may be explained by the 5-HT2BR antagonistic properties of cyproheptadine, since selective blocking of this receptor also prevented isoproterenol- and angiotensin II-induced cardiac hypertrophy (14, 19). In clinical practice, ergotamine-derived dopamine agonists have not been associated with LV dysfunction (21). This apparent contradiction might be due to the relative high dosage of pergolide injected in these animals (21). Also, body weights in cyproheptadine-treated animals were lower than in control animals, which was not expected (11). This could be due to the intraperitoneal administration of cyproheptadine, which might have led to a de-
creased intestinal absorption of nutrients in these rats by its anticholinergic effect. This administration route was chosen to give a corrected dosage at the same time. Although the cyproheptadine-treated animals had lower body weights, this did not influence valvular function and morphology, which confirms the usefulness of this rat model for studying drug-induced valvular heart disease.

We have to acknowledge the limitation that no separate control groups for each vehicle were studied. However, in our previous study (3), neither injections with 10% ethanolic solution (pergolide vehicle) nor injections with physiological saline led to valvular regurgitations or thickening. Moreover, in a pilot study (dose finding for cyproheptadine and DMSO), we did not observe valvular toxicity in rats only receiving DMSO. Also, DMSO is considered a relative safe vehicle with a low or no toxicity profile. In agreement, no valvular toxicity was found in the cyproheptadine-treated group (containing DMSO).

Cyproheptadine is a cheap, safe, off-patent, and widely available drug currently mainly used for its antihistaminic, antiallergic properties (20). It also inhibits several 5-HT receptors (33), which is useful for the symptomatic treatment of metastatic carcinoid syndrome (18). It has even been used for the treatment of neuroleptic-induced akathisia because of its anticholinergic properties (9). Therefore, cyproheptadine might be useful as a prophylactic agent for pergolide-induced valvular heart disease, all the more so, because it lacks and even counteracts the extrapyramidal side effects induced by atypical antipsychotic drugs (3). In addition, the combination of pergolide and cyproheptadine is often used for the treatment of Cushing disease in horses (27).

The findings of this study may have important implications for the practical use of serotonergic compounds such as anti-obesity drugs, amphetamine derivatives [i.e., 3,4-methylenedioxymethamphetamine ("Ecstasy")], antimigraine drugs (ergotamine), and Parkinson’s disease (pergolide and cabergolide) treatments. These drugs have some unique therapeutic effects but have been associated with valvular heart disease (2, 26, 31). The simultaneous use of these drugs with a 5-HT antagonist, such as cyproheptadine, may prevent the develop-

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**Fig. 4.** *Top:* glycosaminoglycan (GAG) and collagen distribution (alcian blue and Masson’s trichrome stainings, respectively) for aortic and mitral valves in the different treatment groups. The increase in the GAG-to-collagen ratio was most pronounced in the mitral valve of the pergolide-treated group. Scale bar = 100 μm. Magnification: ×40. *Bottom:* absence of fibrosis in the LV with increased myocyte thickness in the pergolide-treated group. Scale bar = 50 μm. Magnification: ×200. *P* < 0.05; **P** < 0.01.
ment toward toxic valvulopathy. Moreover, by blocking the 5-HT receptor of valvular fibroblasts, further deterioration of existing drug-induced valvular heart disease might be stopped and perhaps regression may occur. In that way, it might facilitate the regression of valvular heart disease even after removal of the causal drug. Of course, these hypotheses need to be confirmed in prospective clinical trials.

In conclusion, we found that cyproheptadine prevented pergolide-induced valvulopathy in a rat model, which was associated with a reduced number of 5-HT2B-positive valvular cells. These findings may have important clinical implications for treatments with serotonergic drugs.

REFERENCES


