Behavioural consequences of selective activation of 5-HT receptor subtypes
Berendsen, Hermanus Henricus Gerardus

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Chapter 6

A peripheral $5\text{-HT}_{1D}$-like receptor involved in serotonergic induced hindlimb scratching in rats

SUMMARY

The pharmacological characteristics of hindlimb scratching induced by serotonergic compounds were studied. We conclude that hindlimb scratching induced by serotonergic compounds is mediated by a serotonin_1D (5-HT_{1D}) or 5-HT_{1D}-like receptor outside the blood-brain barrier because hindlimb scratching could be induced by s.c. injection of 5-methoxytryptamine (5-MeOT), 5-carboxamidotryptamine (5-CT), bufotenine, 5-hydroxytryptamine (5-HT) and tryptamine. These compounds have high affinity for 5-HT_1A and 5-HT_{1D} receptors. The 5-HT_1A receptor agonist 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT), the 5-HT_{1C} receptor agonist MK 212, and the mixed 5-HT_{1C}/5-HT_{2} receptor agonists (dl)-1-(2,5 dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and quipazine did not induce hindlimb scratching. Rather, the latter compounds attenuated 5-MeOT induced hindlimb scratching. The 5-HT releasing compounds fenfluramine and p-chloroamphetamine (PCA) inhibited whereas the 5-HT re-uptake inhibitors fluvoxamine and indalpine potentiated 5-MeOT-induced hindlimb scratching. 5-MeOT-induced hindlimb scratching could be inhibited dose dependently by the α_2-adrenoceptor blockers yohimbine and rauwolscine, which also have high affinity for 5-HT_{1D} receptors, whereas the α_2-adrenoceptor blocker piperoxan only weakly counteracted hindlimb scratching. Haloperidol, apomorphine, morphine, clonidine and methiothepin strongly attenuated hindlimb scratching, atropine, naloxone and ICS 205930 attenuated it weakly whereas domperidone, methylatropine and mepyramine were inactive in doses up to 10 mg/kg. Hindlimb scratching induced by 5-MeOT was potentiated by the 5-HT receptor antagonists metergoline, methysergide, mesulergine, mianserin, ritanserin and xylamidine. Hindlimb scratching was not induced by i.c.v. injection of 5-MeOT. This lack of i.c.v. activity of 5-MeOT and the effect of xylamidine suggests that the site of action for induction of hindlimb scratching is outside the blood-brain barrier.

6.1 INTRODUCTION

Hindlimb scratching or reciprocal hindlimb scratching in mice has been described after intraperitoneal administration of mescaline and some related dimethoxy amphetamines (Kulkarni, 1973), intracranial injection of substance P and somatostatin (Dobry et al., 1981), intracerebroventricular (i.c.v.) injection of neurohypophyseal hormones (Meisenberg, 1981; 1982) or after intrathecal administration of pilocarpine (Scott et al., 1987). In rats, scratching behaviour has been observed after i.c.v. injection of bombesin, bombesin-like peptides, somatostatin and substance P (Van Wimersma Greidanus et al., 1985; 1987; Negri, 1986; Van Wimersma Greidanus and Maigret, 1988). Recently we noticed that rats showed excessive hindlimb scratching after subcutaneous injection of 5-methoxytryptamine (5-MeOT). 5-MeOT has high affinity for 5-HT receptors (Hoyer, 1989). It is thus possible that this 5-MeOT-induced hindlimb scratching is mediated by serotonergic receptors. It is generally accepted that the 5-HT receptor population can be divided into 5-HT_1 and 5-HT_2 receptor subpopulations on the basis of their affinity for 5-HT and spiperone, respectively (Peroutka and Snyder,
1979). The 5-HT₁ receptors can be subdivided further into 5-HT₁ₐ, 5-HT₁₇, 5-HT₁₆ and 5-HT₁₀ receptor subtypes (Pedigo et al., 1981; Schnellmann et al., 1984; Pazos et al., 1984; Heuring and Peroutka, 1987; Waelder et al., 1988 a,b; Hoyer et al., 1988). It has been possible to ascribe a number of symptoms of the 5-HT syndrome to selective activation of one of these 5-HT receptor subtypes. There is evidence to suggest that selective activation of 5-HT₁ₐ receptors results in the appearance of lower lip retraction (Berendsen et al., 1989), selective activation of 5-HT₁₆ receptors results in the appearance of penile erection (Berendsen et al., 1990) and activation of the 5-HT₂ receptors results in the appearance of head shakes (Yap and Taylor, 1983). If hindlimb scratching induced by 5-MeOT is mediated by a serotonergic mechanism, it is of interest to know which 5-HT receptor subtype(s) might be involved.

In this study we describe the results of a series of experiments showing that 5-HT₁₀ or 5-HT₁₀-like receptors might be involved in drug induced hindlimb scratching after systemic administration.

6.2 MATERIALS AND METHODS

6.2.1 Animals

Naive male wistar rats (Cpb:WU, Harlan Sprague Dawley, Zeist, The Netherlands) weighing 140 - 200 g were used. The animals were housed in white PVC cages (40x40x18 cm) with a wire mesh lid, 5 animals per cage, under a controlled 12 h light-dark cycle, with lights on at 6.00 a.m. The rats were allowed free access to standard food pellets and tap water.

6.2.2 Procedure

All experiments were performed between 9.30 and 14.30 h in a quiet experimental room. Ten animals were scored at the same time, and each treatment group was equally represented in each observation run. Successive runs were performed until the results of 8 animals per treatment were obtained. Treatments were randomized over the animals within a run. After injection of an agonist, the rats were placed in small perspex observation cages (7.5x18x30 cm). A mirror was placed behind these cages to allow all-round observation of the rats.

I.c.v. injections of 5-MeOT were made via guide cannulas (diameter 0.3 mm) into the right lateral ventricle. The cannulas were placed at least one week before the test. The rats were anaesthetised with sodium pentobarbital (60 mg/kg i.p.) and placed in a stereotaxic frame. The cannulas were placed at the coordinates 0.8 mm posterior to the bregma, 1.5 mm lateral to the midline and 3 mm below the dorsal surface of the skull according to the atlas of Paxinos and Watson (1986). The injection needle was 1 mm longer than the guide cannula. Hindlimb scratching was scored from 5 till 30 min after
agonist treatment by scoring the presence or absence of scratching every 30 s; with this
time sampling method the maximum score was 5. For the time response curve for
5-MeOT-induced scratching, the rats were injected with 5-MeOT 1 or 5 mg/kg and
observed from 5 till 30, 35 till 60, 65 till 90 and from 95 till 120 min after injection.

In the interaction studies 5 mg/kg s.c. of 5-MeOT was used to induce scratching.
The direct 5-HT agonists were injected simultaneously with 5-MeOT whereas the other
compounds were injected s.c. 30 min before 5-MeOT.

6.2.3 DRUGS AND SOLUTIONS

The following drugs were used: apomorphine HCl (O.P.G., the Netherlands); atropine sulphate (Nogepha); atropine methylbromide and bufotenine from Sigma; clonidine HCl (synthesised in the chemical Research & Development Laboratories of Organon); 5-carboxamidotryptamine (5-CT), (dl)-1-(2,5 dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI) and 8-hydroxy-2-(di-n-propylamino)tetralin HBr (8-OH-DPAT) from Research Biochemicals Inc. (RBI); domperidone (Motilium®) and haloperidol (Haldol®) from Janssen Pharmaceuticals; fenfluramine HCl (Servier); 5-hydroxytryptamine methane sulfonate (5-HT; Merck); indalpine (Pharmuka); methysergide maleate and 3-tropanyl-indole-3-carboxylate (ICS 205930; Sandoz); mepyramine HCl (Société Parisienne d’ Expansion Chimique); metergoline (Farmitalia); 5-methoxytryptamine (5-MeOT; Aldrich); metoprolol tartrate (Hassle); mianserin HCl (Organon International); 2 chloro-6-(1-piperazinyl) pyrazine monohydro chloride (MK 212; Merck, Sharpe and Dohme); quipazine maleate (Miles Laboratories); rauwolscine (Carl Roth); tryptamine HCl (Fluka); yohimbine HCl (ACF Chemie Farma) and xylamidine tosylate (Wellcome Research Laboratories).

Haloperidol was diluted from 5 mg/kg Haldol® ampoules to the required
concentrations in sterile saline. Apomorphine was dissolved in saline containing 0.5 mg
of ascorbic acid and 0.5 mg of mannitol per mg of apomorphine. Metergoline, domperidone and xylamidine were suspended in an aqueous solution of 5% Mulgofen
(EL 719®, GAF Corp.) and 0.9% NaCl. All other drugs were dissolved in sterile saline
solution. All drug solutions or suspensions were freshly prepared and were injected s.c.
into the loose skin at the back of the neck.

A volume of 5 ml/kg body weight was used. Control animals received an
equivalent volume of vehicle. When drug solutions were made up from the salt of the compound, the doses refer to the weight of the salt.

6.2.4 STATISTICS

The results are expressed as the mean score per group ± S.E.M. The statistical
significance of the drug effects was determined with the Kruskal-Wallis ANOVA
followed by the Mann-Whitney U-test in the interaction studies. ID_{50} values were
calculated by the linear regression analysis according to the method of Litchfield and Wilcoxon (1949).

6.3 RESULTS

6.3.1 Induction of hindlimb scratching

Untreated, or placebo treated rats placed in the observation cages used in these experiments hardly scratched, the highest score reached during the 25 min observation period was $3.0 \pm 0.5$. However, within a few minutes after subcutaneous injection of 5-MeOT the rats started to make scratching movements with their hindlimbs.

![Graph](image)

Figure 1: Time-response curve for 5-MeOT induced scratching. Scratching behaviour was scored during 25 min of each half hour, i.e., from 5 - 30 min, 35 - 60 min, 65 - 90 min and from 95 - 120 min after 5-MeOT injection. These 25 min were divided in 2 periods of 12.5 min, and the mean scores of these periods $\pm$ S.E.M. are plotted in the fig. Eight animals per group were used.

- ▲ ▲ placebo;
- ● ● 5-MeOT 1 mg/kg;
- ○ ○ 5-MeOT 5 mg/kg.
Table 1. Induction of hindlimb scratching. Hindlimb scratching was scored from 5 till 30 min after s.c. injection of the compounds by using a time sampling method. Eight animals per group were observed.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg s.c.)</th>
<th>Mean hindlimb scratching score ± S.E.M.</th>
<th>Kruskal-Wallis</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-MeOT</td>
<td>0</td>
<td>0.5 ± 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>1.0 ± 0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.22</td>
<td>2.6 ± 0.7</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>0.46</td>
<td>12.0 ± 2.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>25.5 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>5-MeOT</td>
<td>0</td>
<td>1.3 ± 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>18.4 ± 3.1</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>25.6 ± 3.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.6</td>
<td>30.6 ± 2.6</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>32.3 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>Bufotenine</td>
<td>0</td>
<td>3.0 ± 0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>12.6 ± 2.5</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>16.6 ± 2.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.6</td>
<td>26.8 ± 2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>25.4 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>5-CT</td>
<td>0</td>
<td>1.0 ± 0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.046</td>
<td>6.5 ± 3.3</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>7.8 ± 1.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.22</td>
<td>21.8 ± 3.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.46</td>
<td>26.5 ± 2.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>27.4 ± 2.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>31.4 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>5-HT</td>
<td>0</td>
<td>2.3 ± 0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>11.3 ± 2.0</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>19.8 ± 1.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.6</td>
<td>18.4 ± 2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>6.9 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>Tryptamine</td>
<td>0</td>
<td>1.5 ± 0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.6</td>
<td>5.9 ± 1.9</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>11.8 ± 2.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22.0</td>
<td>11.1 ± 1.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>46.0</td>
<td>14.6 ± 2.5</td>
<td></td>
</tr>
</tbody>
</table>

The maximal response was reached after about 30 min, while the duration of the response depended on the dose given (fig. 1). The skin or fur is not always touched during these scratching movements, and this is termed hindlimb scratching. Hindlimb scratching was measured from 5 till 30 min after 5-MeOT treatment in all interaction studies. 5-MeOT induced hindlimb scratching in a dose-dependent manner, 0.1 mg/kg being inactive and 0.22 mg/kg inducing a small but significant (P < 0.05) increase in scratching (table 1). Subcutaneous injection of 5-CT, bufotenine, serotonin and
Figure 2: Effect of yohimbine, rauwolscine and piperoxan on 5-MeOT (5 mg/kg)-induced hindlimb scratching. The columns represent the mean scratching scores of 8 animals, as measured from 5 till 30 min after 5-MeOT treatment. The compounds were injected 30 min before 5-MeOT. The vertical bars represent the S.E.M.

* P<0.05; ** P<0.01; *** P<0.001

tryptamine also induced hindlimb scratching (table 1). The response to 5-CT was dose-dependent and a mean score ± S.E.M. of 31.4 ± 2.9 was reached after 2.2 mg/kg. At 0.1 mg/kg, 5-CT hardly induced hindlimb scratching whereas at 0.22 mg/kg marked hindlimb scratching was seen. A maximum score of 26.8 ± 2.0 was reached with 4.6 mg/kg bufotonine and then the response levelled off.

The scores for serotonin and tryptamine were much lower. After serotonin 2.2 mg/kg a score of 19.8 ± 1.6 and after tryptamine (46 mg/kg) a score of 14.6 ± 2.5 was reached; doses up to 4.6 mg/kg tryptamine hardly induced hindlimb scratching. The dose-response curve for serotonin had an inverted U shape with a peak effect after 2.2 mg/kg. Hindlimb scratching was not seen after i.c.v. injection of 5-MeOT in doses up to 100 µg/rat. No other compound used in this study induced hindlimb scratching. Besides scratching, 5-CT treated rats showed weak lower lip retraction, and 5-HT (10 mg/kg)-
Figure 3: The effect of some indirect 5-HT agonists on 5-MeOT (5 mg/kg)-induced hindlimb scratching. A: 5-HT releasing compounds, B: 5-HT reuptake inhibitors. The mean scratching scores from 8 animals per group, measured from 5 till 30 min after 5-MeOT treatment, are given. The compounds were injected 30 min before 5-MeOT. The vertical bars represent the S.E.M. * P<0.05; ** P<0.01; *** P<0.001

Table 2.
Effect of a variety of compounds on 5-MeOT (5 mg/kg s.c.)-induced hindlimb scratching. ID$_{50}$ values are given. Hindlimb scratching was scored from 5 till 30 min after 5-MeOT injection. Pretreatment times are given in parentheses; all drugs were s.c. injected in groups of 8 animals.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>ID$_{50}$ in mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-OH-DPAT</td>
<td>(0) 0.05</td>
</tr>
<tr>
<td>MK 212</td>
<td>(0) 0.6</td>
</tr>
<tr>
<td>DOI</td>
<td>(0) 0.14</td>
</tr>
<tr>
<td>Quipazine</td>
<td>(0) 0.3</td>
</tr>
<tr>
<td>ICS 205930</td>
<td>(30) 10</td>
</tr>
<tr>
<td>Methiothepin</td>
<td>(30) 0.2</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>(30) 0.05</td>
</tr>
<tr>
<td>Apomorphine</td>
<td>(0) 0.07</td>
</tr>
<tr>
<td>Clonidine</td>
<td>(0) 0.015</td>
</tr>
<tr>
<td>Morphine</td>
<td>(30) 0.6</td>
</tr>
<tr>
<td>Naloxone</td>
<td>(30) &gt;2.2</td>
</tr>
<tr>
<td>Atropine</td>
<td>(30) &gt;4.6</td>
</tr>
<tr>
<td>Methylatropine</td>
<td>(30) inactive up to 10 mg/kg</td>
</tr>
<tr>
<td>Domperidone</td>
<td>(30) inactive up to 10 mg/kg</td>
</tr>
<tr>
<td>Mepyramine</td>
<td>(30) inactive up to 10 mg/kg</td>
</tr>
</tbody>
</table>
Table 3. Effect of 5-HT antagonists on 5-MeOT (5 mg/kg s.c.)-induced hindlimb scratching. Compounds were injected 30 min before 5-MeOT

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose mg/kg s.c.</th>
<th>Mean hindlimb scratching score ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylamidine tosylate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>29.5 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>39.1 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>0.22</td>
<td>42.5 ± 0.8c</td>
<td></td>
</tr>
<tr>
<td>0.46</td>
<td>42.6 ± 1.9b</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>44.6 ± 0.9c</td>
<td></td>
</tr>
<tr>
<td>Mesulergine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>31.4 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>0.022</td>
<td>37.9 ± 1.8a</td>
<td></td>
</tr>
<tr>
<td>0.046</td>
<td>40.6 ± 0.8b</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>38.5 ± 1.9a</td>
<td></td>
</tr>
<tr>
<td>0.22</td>
<td>38.4 ± 2.2a</td>
<td></td>
</tr>
<tr>
<td>Metergoline</td>
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</tr>
<tr>
<td>0</td>
<td>29.8 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>36.9 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>0.22</td>
<td>39.0 ± 1.0b</td>
<td></td>
</tr>
<tr>
<td>0.46</td>
<td>42.4 ± 1.9b</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>40.4 ± 1.9b</td>
<td></td>
</tr>
<tr>
<td>Methysergide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>25.1 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>39.1 ± 0.7c</td>
<td></td>
</tr>
<tr>
<td>0.22</td>
<td>39.9 ± 1.6c</td>
<td></td>
</tr>
<tr>
<td>0.46</td>
<td>41.0 ± 2.1c</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>37.4 ± 1.8b</td>
<td></td>
</tr>
<tr>
<td>Mianserin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>27.9 ± 3.6</td>
<td></td>
</tr>
<tr>
<td>0.046</td>
<td>33.5 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>37.4 ± 1.9a</td>
<td></td>
</tr>
<tr>
<td>0.22</td>
<td>40.9 ± 1.9b</td>
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</tr>
<tr>
<td>0.46</td>
<td>41.6 ± 1.3a</td>
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<tr>
<td>Ritanserin</td>
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<tr>
<td>0</td>
<td>26.8 ± 3.4</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>32.9 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>0.22</td>
<td>36.5 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>0.46</td>
<td>38.3 ± 1.3a</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>38.3 ± 1.6a</td>
<td></td>
</tr>
</tbody>
</table>

*aP < 0.05; *bP < 0.01; *cP < 0.001 compared to control group

Treated rats showed flat body posture, hypoactivity and cyanosis. Tryptamine-treated rats showed slight ptosis.

6.3.2 Antagonism of 5-MeOT-induced hindlimb scratching

A dose of 5 mg/kg of 5-MeOT was used for the interaction studies. Scratching scores after this dose varied between a mean ± S.E.M. of 22.1 ± 2.7 and 31.4 ± 1.8. Hindlimb scratching induced by 5-MeOT was dose dependently antagonised by
yohimbine and rauwolscine (fig. 2; ID\textsubscript{50} values were 3 and 4 mg/kg, respectively). The effect of piperoxan was variable, at 10 mg/kg an inhibition of 53% was reached. Other compounds that attenuated 5-MeOT-induced scratching were the serotonergic agonists 8-OH-DPAT, MK 212, DOI and quipazine; their ID\textsubscript{50} values are shown in table 2. Scratching was also attenuated by the 5-HT releasing compounds, fenfluramine and PCA (fig. 3).

5-MeOT-induced hindlimb scratching could be inhibited by methiothepin, haloperidol, apomorphine, clonidine and morphine (ID\textsubscript{50} values were 0.2; 0.05; 0.07; 0.015 and 0.6 mg/kg, respectively), whereas ICS 205930, atropine and naloxone were only weakly active (ID\textsubscript{50} values: 10; >2.2 and >4.6 mg/kg, respectively) and domperidone, mepyramine and methylatropine were inactive in doses up to 10 mg/kg (table 2). Lower lip retraction was also seen after 8-OH-DPAT, penile erections after MK 212 and head shakes after DOI and quipazine. The animals were sedated after methiothepin, apomorphine, haloperidol, clonidine and morphine.

### 6.3.3 Potentiation of 5-MeOT-induced hindlimb scratching

Hindlimb scratching increased when the rats were treated with the 5-HT receptor antagonists xylamidine (0.1 - 1.0 mg/kg), mesulergine (0.022 - 0.22 mg/kg), methysergide (0.1 - 1.0 mg/kg), metergoline (0.1 - 1.0 mg/kg), mianserin (0.046 - 0.46 mg/kg) or ritanserin (0.1 - 1.0 mg/kg) 30 min before 5-MeOT was injected (table 3). This potentiation of hindlimb scratching was statistically significant and dose dependent for all these compounds. The 5-HT re-uptake blockers, fluvoxamine and indalpine, also increased 5-MeOT-induced hindlimb scratching (fig. 3).

### 6.4 DISCUSSION

In this study we found that s.c. injection of the 5-HT agonists 5-MeOT, 5-CT, bufotenine, 5-HT and tryptamine induced hindlimb scratching. Hindlimb scratching was not seen after injection of the 5-HT receptor agonists 8-OH-DPAT, MK 212, DOI and quipazine. 5-MeOT, 5-CT, bufotenine, 5-HT and tryptamine all have high affinity for 5-HT\textsubscript{1A} and 5-HT\textsubscript{1D} receptors (Hoyer, 1989; table 4). 8-OH-DPAT has also high affinity for 5-HT\textsubscript{1A} receptors but hardly binds to 5-HT\textsubscript{1D} receptors. As 8-OH-DPAT did not induce hindlimb scratching, 5-HT\textsubscript{1A} receptors are not likely to be involved in this response. It is thus attractive to suggest that hindlimb scratching mediated by serotonergic compounds is related to an agonistic effect of these compounds on 5-HT\textsubscript{1D}- or 5-HT\textsubscript{1D}-like receptors. 5-HT\textsubscript{1D} receptors have been found in the basal ganglia and the substantia nigra in bovine, pig and human brains (Heuring and Peroutka, 1987; Waeber et al., 1988 a,b), whereas the same areas in the rat are rich in 5-HT\textsubscript{1B} receptors (Pazos and Palacios, 1985). Herrick-Davies and Titeler (1988), however, found a 5-HT receptor in the rat brain with a pharmacology similar to that of
Table 4. Affinity of several 5-HT agonists used in this study for the different 5-HT receptor subtypes

<table>
<thead>
<tr>
<th>Drugs</th>
<th>5-HT₁A</th>
<th>5-HT₁B</th>
<th>5-HT₁C</th>
<th>5-HT₁D</th>
<th>5-HT₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-MeOT</td>
<td>8.0</td>
<td>6.4</td>
<td>7.6</td>
<td>8.4</td>
<td>5.5</td>
</tr>
<tr>
<td>5-CT</td>
<td>9.5</td>
<td>8.3</td>
<td>6.2</td>
<td>8.6</td>
<td>4.7</td>
</tr>
<tr>
<td>Bufotenine</td>
<td>7.6</td>
<td>6.0</td>
<td>7.2</td>
<td>8.1</td>
<td>6.4</td>
</tr>
<tr>
<td>5-HT</td>
<td>8.5</td>
<td>7.6</td>
<td>7.5</td>
<td>8.4</td>
<td>5.5</td>
</tr>
<tr>
<td>Tryptamine</td>
<td>6.8</td>
<td>5.0</td>
<td>7.3</td>
<td>7.1</td>
<td>6.0</td>
</tr>
<tr>
<td>8-OH-DPAT</td>
<td>8.7</td>
<td>4.2</td>
<td>5.2</td>
<td>5.9</td>
<td>5.0</td>
</tr>
<tr>
<td>Quipazine</td>
<td>5.5</td>
<td>6.5</td>
<td>6.7</td>
<td>5.9</td>
<td>6.2</td>
</tr>
<tr>
<td>DOI¹</td>
<td>5.6</td>
<td>5.9</td>
<td>7.7</td>
<td></td>
<td>7.8</td>
</tr>
<tr>
<td>MK 212²</td>
<td>5.3</td>
<td>5.0</td>
<td>6.2</td>
<td></td>
<td>4.7</td>
</tr>
</tbody>
</table>

Affinity values are from Hoyer, 1989. ¹) Values are calculated from Titeler et al., 1988. ²) Values are from Hoyer, 1988.

the 5-HT₁D receptor detected in bovine brain by Heuring and Peroutka (1987). Thus it seems that both 5-HT₁D and 5-HT₁B receptors may be present in the rat CNS.

Serotonergically induced hindlimb scratching, however, seems to be mediated by a receptor system that is localised outside the blood-brain barrier. Hindlimb scratching could be induced by systemic but not i.c.v. injection of 5-MeOT. The 100 µg/rat dose given i.c.v. is approximately equal to a dose of 0.5 mg/kg. Such a dose, if given s.c., induces scratching. Furthermore, 5-MeOT-induced hindlimb scratching could be potentiated by xylamidine, a compound that does not cross the blood-brain barrier (Fuller et al., 1986). In the periphery, 5-HT₁D- or 5-HT₁D-like receptors might be present in the carotid vascular bed and be involved in the pathophysiology of migraine. GR 43175 and serotonin, compounds that do not cross the blood-brain barrier and which have a high affinity for 5-HT₁D receptors (Peroutka and McCarthy, 1989; Hoyer, 1989), appear to be highly effective in suppressing acute migraine attacks (Saxena and Ferrari, 1989; Doenicke et al., 1988).

Serotonergically mediated scratching is distinguished from peptide-induced scratching in rats by the site of action. Peptidergic scratching has only been seen after i.c.v. application of the compounds and is therefore centrally mediated (Van Wimersma Greidanus et al., 1985, 1987; Negri, 1986; Van Wimersma Greidanus and Maigret, 1988).

5-MeOT-induced hindlimb scratching was dose dependently inhibited by the α₂ adrenoceptor blockers yohimbine and rauwolscine. Both compounds have, besides their α₂ blocking properties, high affinity for 5-HT₁D receptors (Hoyer, 1989). It is thus attractive to speculate that the effect of these compounds on hindlimb scratching is due to their 5-HT₁D antagonistic properties. However, a non-specific effect of these compounds cannot be excluded since the α₂-adrenoceptor blocker piperoxan also had an
effect on hindlimb scratching. Both yohimbine and rauwolscine caused sedation at a dose of 4.6 mg/kg, which might contribute to the inhibition of hindlimb scratching. Sedation may also be the best explanation for the inhibiting effect of haloperidol on hindlimb scratching since this compound hardly binds to 5-HT1D receptors (Hoyer, 1989) and an effect mediated by peripheral dopamine receptor blockade is unlikely since hindlimb scratching was not affected by domperidone, a dopamine receptor blocker that does not pass the blood brain barrier and does not cause sedation. The dopaminergic agonist apomorphine strongly and dose dependently inhibited 5-MeOT-induced hindlimb scratching. Both haloperidol and apomorphine caused sedation in the doses tested, as did morphine, clonidine, ICS 205930 and methiothepin. It is thus possible that serotonergic-mediated hindlimb scratching is very sensitive to sedation, independent of the underlying transmitter mechanisms. The observation that hindlimb scratching can be overruled by other behavioural disturbances might also be the cause of the biphasic effect of 5-HT, since at 10 mg/kg flat body posture and cyanosis were seen. The small response seen after tryptamine might also be caused by non-specific side effects (ptosis), but it should also be noted that, of the compounds tested, tryptamine has the lowest affinity for 5-HT1D receptors (table 4).

5-MeOT-induced hindlimb scratching was dose dependently inhibited by the 5-HT1A receptor selective agonist 8-OH-DPAT, the 5-HT1C receptor agonist MK 212 and the mixed 5-HT1C/5-HT2 receptor agonists DOI and quipazine. We have seen before that lower lip retraction induced in rats by activation of central 5-HT1A receptors can be attenuated by concomitant activation of 5-HT1C and/or 5-HT2 receptors (Berendsen et al., 1989) and that penile erections induced by activation of central 5-HT1C receptors can be attenuated by concomitant activation of 5-HT1A or 5-HT2 receptors (Berendsen et al., 1990). A similar functional influence may take place between 5-HT1D or 5-HT1D-like receptors and 5-HT1A, 5-HT1C and 5-HT2 receptor subtype-mediated events. It should be noted however, that a compound like 5-CT, which has high affinity for both 5-HT1A and 5-HT1D binding sites, induced lower lip retraction, be it weakly, and hindlimb scratching, whereas 8-OH-DPAT strongly inhibited hindlimb scratching. The efficacies of the compounds for 5-HT1A and 5-HT1D receptors might be very important in this respect.

The effects of the indirect 5-HT receptor agonists are puzzling. The 5-HT releasing compounds fenfluramine and PCA attenuated hindlimb scratching whereas the 5-HT-re-uptake inhibitors indalpine and fluvoxamine potentiated hindlimb scratching. In both cases the synaptic availability of 5-HT is increased, and both 5-HT-releasing compounds and 5-HT-re-uptake inhibitors induce 5-HT1C receptor mediated penile erections (Berendsen and Broekkamp, 1987; Berendsen et al., 1990). As yet we do not have an explanation for this difference.

5-MeOT-induced hindlimb scratching was potentiated by the 5-HT1C/5-HT2 receptor antagonists xylamidine, metergoline, methysergide, mesulergine, mianserin and ritanserin. The potentiating effect of these 5-HT receptor antagonists cannot simply be explained by a facilitating effect due to blockade of other 5-HT receptor subtypes, because metergoline and methysergide have a high affinity for 5-HT1C, 5-HT2 and 5-HT1D receptors (Hoyer, 1989). These compounds may, however, act as agonists at the
receptor mediating hindlimb scratching. In line with this idea are the findings of Saxena (1974) and Humphrey et al. (1990) that methysergide acts as an agonist at peripheral 5-HT receptors by selectively causing carotid vasoconstriction, an effect also caused by 5-HT. The 5-HT receptor antagonist methiothepin inhibited 5-MeOT-induced hindlimb scratching. This compound has substantial 5-HT_{1D} receptor antagonistic properties (Hoyer and Middlemiss, 1989) but is also a strong dopamine receptor antagonist and causes sedation. It is not clear which of these properties was responsible for the attenuating effect on hindlimb scratching. Hindlimb scratching induced by systemic injection of serotonergic compounds seems to be mediated by 5-HT_{1D}-like receptors outside the blood-brain barrier. Some of the synaptic events involved remain to be clarified.

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