Chapter 7

Age dependence of 5-Methoxytryptamine-induced hindlimb scratching in rats

SUMMARY
The influence of age on hindlimb scratching in rats induced by the serotonergic agonist 5-methoxytryptamine (5-MeOT) was studied. 5-MeOT-induced scratching was strongest in 30 day old rats and least in rats of 90 days. Sex hormonal influences do not play a role in these differences since treatment of young male rats with testosterone propionate did not change the scratching response. In female rats the same age dependence was found as for males.

7.1 INTRODUCTION
Hindlimb scratching in rats has been reported after intracerebroventricular (i.c.v.) injection of bombesin, bombesin-like peptides, somatostatin and substance P (Van Wimersma Greidanus et al., 1987; Negri, 1986; Van Wimersma Greidanus and Maigret, 1988). Recently we found that several serotonergic compounds also induce excessive hindlimb scratching but only after systemic injection and not after i.c.v. injection suggesting a peripheral mechanism of action (Berendsen and Broekkamp, 1991). This hindlimb scratching induced by serotonergic compound is possibly mediated by a 5-HT1D-like receptor as it could be induced by compounds which have a high affinity for the 5-HT1D receptor, such as 5-carboxamidotryptamine (5-CT), 5-methoxytryptamine (5-MeOT), bufotenine, 5-hydroxytryptamine (5-HT) and tryptamine but not by 8-OH-DPAT. During these studies it was observed that this serotonin mediated hindlimb scratching appeared to be more intense in younger rats. In this study we confirm a strong age dependence of 5-MeOT induced hindlimb scratching.

7.2 MATERIALS AND METHODS

7.2.1 Animals
Naive male or female Wistar rats (Cpb: WU, Harlan Sprague Dawley, Zeist, The Netherlands) were used. The body weights of the male rats were 90 - 110 g, 180 - 210 g, 275 - 300 g and 370 - 400 g and this corresponds to an age of approximately 30, 45, 60 and 90 days. The female rats were approximately 30 and 90 days of age.

The rats were housed in white PVC cages (40 x 40 x 18 cm) with a wire mesh lid, 5 animals per cage, under controlled 12 h light-dark cycle, with lights on at 6.00 a.m. The rats had free access to standard food pellets and tap water. Each rat was used only once.
7.2.2 Procedure

The experiments were performed between 9.30 and 12.30h in a quiet experimental room. Hindlimb scratching was induced by 5-methoxytryptamine (5-MeOT; Aldrich) or bufotenine (Sigma). Immediately after s.c. injection with 5-MeOT the rats were placed in individual small perspex observation cages (7.5 x 18 x 30 cm). A mirror was placed behind these cages to facilitate and allow allround observation.

Treatments were randomized over all animals within an experiment. Groups of 10 animals were scored at the same time until 8 animals were observed for each treatment group. Hindlimb scratching was scored from 5 till 30 min after s.c. injection of 5-MeOT as described before (Berendsen and Broekkamp, 1991): the rats were scored for having scratched (+) or not (-) during each 30 s in this period. Using this method a maximal total score of 51 could be reached. Bufotenine-induced scratching was only measured in 90 day old rats. In the test with testosterone, testosterone propionate (Organon) 1 mg/kg/day was given for 3 days preceding the actual experiment. 5-MeOT and bufotenine were dissolved in a sterile saline solution and these solutions were freshly prepared for each experiment. The compound was injected in a dose volume of 5 ml/kg body weight. Testosterone propionate was dissolved in arachis oil and injected in a dose volume of 1 ml/kg body weight. Control animals received an equivalent volume of saline or arachis oil. All injections were made s.c. in the loose skin at the back of the neck.

Scratching score

![Graph](image)

Figure 1: 5-Methoxytryptamine (5-MeOT) induced hindlimb scratching in male rats of different ages. The points represent mean scores ± S.E.M. Eight animals per group were used.
7.3 RESULTS

The dose response curves for hindlimb scratching induced by 5-MeOT in male rats of different ages are presented in fig. 1. Induction of hindlimb scratching by 5-MeOT was dose dependent up to 2.2 mg/kg of the compound and thereafter reached a plateau in rats of all ages. The intensity of hindlimb scratching was strongly dependent on the age of the rats. Hindlimb scratching score was highest in the 30 day old rats followed by the 45 day and the 60 day old rats, whereas in rats of 90 days hardly an increase in hindlimb scratching was seen. Other behavioural changes were not observed. Also after bufotenine (1 - 10 mg/kg) the hindlimb scratching in 90 day old rats was hardly increased. Their mean scratching score ± S.E. was 4.0 ± 1.7.

In the experiments with female rats the same age dependence in hindlimb scratching was found. After 2.2 mg/kg of 5-MeOT the mean scores ± S.E. were 39.0 ± 1.1 and 13.3 ± 1.5 for 30 and 90 day old rats respectively.

In 30 day old male rats that were treated with testosterone propionate (1 mg/kg/day) during 3 days preceding the scratching test no difference was found in scratching score after 5-MeOT (2.2 mg/kg) if compared to placebo pretreated rats. Scratching scores were 38.1 ± 2.1 and 35.8 ± 2.5 respectively for placebo and testosterone pretreated rats.

7.4 DISCUSSION

This study shows that induction of hindlimb scratching by the serotonin agonist 5-MeOT is most pronounced in juvenile rats and disappears when the rats grow older. Also bufotenine could not induce hindlimb scratching in 90 day old rats, whereas it has been shown to be active in younger rats (Berendsen and Broekkamp, 1991). It is unlikely that these differences in hindlimb scratching are due to differences in the rate of metabolism of 5-MeOT since the dose response curves in rats of all ages tested plateaued above a dose 2.2 mg/kg of 5-MeOT. Hindlimb scratching induced by serotonergic compounds in rats is probably mediated by a 5-HT1D-like receptor (Berendsen and Broekkamp, 1991). The 5-HT1D receptor has been found in the brain of pig, calf and human (Heuring and Peroutka, 1987; Waeber et al., 1988), but not in rats. In the rats the 5-HT1B receptor is found in the same brain areas in which the 5HT1D receptor has its highest density in the other species (Heuring et al., 1986). Moreover both the 5-HT1B and 5HT1D receptors are coupled to the same second messenger system. It has been reported that 5-HT1B and 5-HT1D receptors mediate inhibition of forskolin-stimulated adenylate cyclase activity (Bouhelal et al., 1988; Hoyer and Schoeffler, 1988). Herrick-Davies and Titeler (1988) found a receptor in rats with “similar pharmacology to the 5-HT1D site detected in bovine brain by Heuring and Peroutka” (1987). This suggests that both the 5-HT1B and 5-HT1D receptors might be present in the rat.

If hindlimb scratching is mediated by the 5-HT1D receptor than it could be that the density of these receptors is higher in younger animals and that this receptor gradually disappears when the rats grow older. Binding studies were done in older rats and mice.
and this could be a reason that such a receptor has not been detected as yet in these species. The 5-HT$_{1D}$ receptor has been detected in binding studies in young bovine: the calf. There are no reports on binding in the adult cow. Serotonergic induced hindlimb scratching is mediated outside the blood brain barrier as it can be induced by s.c. administration of serotonin itself and s.c. but not i.c.v. administration of 5-MeOT (Berendsen and Broekkamp, 1991). As binding studies generally use brain tissue, it may be that this 5-HT$_{1D}$ receptor is not observed because it is only present in the periphery.

The strong age dependency of a behaviour which is not related to sexual behaviour or to parent-infant contacts is surprising. Apart from playfulness we have not found reports on such strong behavioural changes in the development to maturity.

A relation to increase in sexual hormones was not found as administration of testosterone to 30 day old rats, did not suppress 5-MeOT induced scratching and the age dependency was also found in female rats. Apart from the aspects on development of behaviour these findings should influence research on serotonin receptor characterization in different species.

REFERENCES


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