Enhanced sensitivity of postsynaptic serotonin-1A receptors in rats and mice with high trait aggression

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Received 15 December 2000; received in revised form 13 April 2001; accepted 24 May 2001

Abstract

Individual differences in aggressive behaviour have been linked to variability in central serotonergic activity, both in humans and animals. A previous experiment in mice, selectively bred for high or low levels of aggression, showed an up-regulation of postsynaptic serotonin-1A (5-HT1A) receptors, both in receptor binding and in mRNA levels, in the aggressive line [Brain Res 736 (1996) 338]. The aim of this experiment was to study whether similar differences in 5-HT1A receptors exist in individuals from a random-bred rat strain, varying in aggressiveness. In addition, because little is known about the functional consequences of these receptor differences, a response mediated via postsynaptic 5-HT1A receptors (i.e., hypothermia) was studied both in the selection lines of mice and in the randomly bred rats. The difference in receptor binding, as demonstrated in mice previously, could not be shown in rats. However, both in rats and mice, the hypothermic response to the 5-HT1A agonist alnespirone was larger in aggressive individuals. So, in the rat strain as well as in the mouse lines, there is, to a greater or lesser extent, an enhanced sensitivity of postsynaptic 5-HT1A receptors in aggressive individuals. This could be a compensatory up-regulation induced by a lower basal 5-HT neurotransmission, which is in agreement with the serotonin deficiency hypothesis of aggression. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Aggression; Serotonin; Receptor binding autoradiography; Hypothermic response; Wild-type Groningen rats; SAL and LAL mice

1. Introduction

Individual differences in aggressiveness have been linked to variability in central serotonergic (5-HT) activity. Numerous studies have shown a negative correlation between the cerebrospinal fluid level of 5-hydroxyindoleacetic acid (5-HIAA), the major metabolite of serotonin, and aggression in humans (for reviews, see Refs. 5,6,13,17,32,34) and primates [12,20,35]. On the other hand, rats selected for low aggressiveness seem to have an increased serotonergic activity, measured by increased tryptophan hydroxylase activity and increased levels of 5-HT and 5-HIAA in several brain areas, compared to wild rats [29]. Although some controversy exists, the general view is that aggressive individuals have a low serotonergic neurotransmission. These observations form the basis for the so-called 5-HT deficiency hypothesis of aggression.

Against this background, two mouse lines, selectively bred for high or low levels of aggression, have been studied. In the aggressive line, an up-regulation of postsynaptic serotonin-1A (5-HT1A) receptors was found in several brain areas, both in receptor binding and in mRNA expression [16]. The question arose whether this is a difference specific for these mouse lines, or a more general characteristic of individuals with high or low trait aggression. Therefore, it was examined if the same difference is present in a nonselected strain of rats, which is characterised by a rich behavioural profile and large interindividual differences in aggressiveness. This paper describes the experiments in which the number of 5-HT1A receptor binding sites in the brains of aggressive and nonaggressive individuals from a randomly bred rat strain was studied, using quantitative autoradiography.

Moreover, one might expect that differences in binding are also reflected in the functionality of this receptor system.
As a model of postsynaptic 5-HT\textsubscript{1A} receptor function, the hypothermic response after administration of a 5-HT\textsubscript{1A} agonist is often used [1,18,36]. In a second experiment, we studied the extent in which aggressive and nonaggressive rats and mice show a hypothermic response to a challenge with the 5-HT\textsubscript{1A} receptor agonist alnespirone.

A complicating factor is that this hypothermic response is thought to be mediated via postsynaptic receptors in rats [1,21,27] but via autoreceptors in mice [2,10,11,19], although this is not indisputably demonstrated. In order to facilitate the interpretation of the results in terms of postsynaptic versus autoreceptor involvement, an additional experiment was performed in mice. S-15535, a drug that acts as agonist on the 5-HT\textsubscript{1A} autoreceptor and as antagonist on the 5-HT\textsubscript{1A} postsynaptic receptor [22–24,30], was administered alone and in combination with alnesprone and the effects on body temperature were measured. When the hypothermic response is mediated via the autoreceptor, one may expect that administration of S-15535 alone will induce hypothermia; but when the response is mediated via the postsynaptic receptor, S-15535 should not induce hypothermia by itself and inhibit the response induced by alnesprone.

2. Materials and methods

2.1. Animals and housing

The rats used in this study were male wild-type Groningen rats (Rattus norvegicus). This strain has been bred for approximately 18 generations in our laboratory and the ancestors were caught in the wild. It was chosen for this study because of the rich social behaviour and the high interindividual variation in genetics, behaviour, and physiology.

Throughout the whole experiment, rats had free access to food and water and were housed under a 12:12-h light–dark cycle, experiments being carried out in the dark phase. From weaning on, at 23 days of age, the rats were housed in groups of six in clear Plexiglas cages (55 × 35 × 20 cm). At 4.5 months of age, rats were tested for offensive aggression in a resident–intruder paradigm. Each rat was housed in a cage (80 × 55 × 40 cm) together with a sterilised female to stimulate territorial behaviour and to prevent social isolation. After a week of habituation, four aggression tests were carried out on consecutive days. For each test, an unfamiliar male conspecific (Wistar) was introduced into the home cage of the experimental rat. The female was removed 30 min in advance. On the first 3 days, only the attack latency time was scored with a maximum test duration of 10 min; the test was terminated shortly after occurrence of an attack. During the fourth test, the full behavioural profile was recorded for 10 min, using The Observer (Noldus Information Technology, Wageningen, the Netherlands, version 3.0). The following behavioural elements were scored: aggressive behaviour (clinch, threat, offensive upright, keep down, chase), social behaviour (investigate opponent, sniff in anogenital region, social groom, mount), explorative behaviour (explore environment, rear), immobility, and grooming (see Ref. [15] for a more detailed description of agonistic behaviour). By means of this series of aggression tests, the rats were characterised as being aggressive, when they attacked in at least three of the tests and showed aggressive behaviour over 50% of the time in the fourth test, and as nonaggressive, when they did not attack at all and showed hardly any aggressive behaviour (less than 10% of the time). Both aggressive and nonaggressive individuals were studied in the experiments described below.

The male mice used in this study were from two lines selectively bred for high or low levels of aggression. The high aggressive mice have a short attack latency (SAL); the low aggressive mice, on the other hand, have a long attack latency (LAL) or do not attack at all. These SAL and LAL lines are in their 60th and 33rd generation, respectively (see Ref. [33] for details). The male mice were housed with a female in clear Plexiglas cages (18 × 12 × 13 cm), in a 12:12-h light–dark cycle, with food and water ad libitum. Experiments were carried out in the dark phase.

2.2. Receptor binding autoradiography

The 5-HT\textsubscript{1A} receptor binding was examined in an autoradiographic study with [\textsuperscript{3}H] 8-OH-DPAT (a full and selective 5-HT\textsubscript{1A} receptor agonist) based on the method described by Pazos and Palacios [28].

Rats, characterised as being aggressive (n = 6) or nonaggressive (n = 5), were decapitated half an hour after the start of the dark phase on the day after the last aggression test. The brains were quickly taken out, frozen on liquid nitrogen, and kept at −80°C. Sections of 20 μm thick were cut at −18°C using a cryostat, mounted on gelatin-coated slides, and stored at −80°C until incubation.

The brain sections were preincubated in 0.17 M Tris–HCl buffer (pH 7.6), containing 4 mM CaCl\textsubscript{2} and 0.01% ascorbic acid, for 3 × 10 min at room temperature. Then the sections were incubated for 1 h at room temperature in a moist environment with 1.5 nM [\textsuperscript{3}H] 8-OH-DPAT (Amersham TRK 850, specific activity: 221 Ci/mmol) in the same buffer as used for preincubation, but also containing 10 μM pargyline. Directly afterwards, they were washed 3 × 90 s in cold (4°C) buffer, 10 s in cold distilled water, and dried. The sections were exposed to a tritium-sensitive hyperfilm (Amersham), together with a tritium standard scale (\textsuperscript{3}H-micro-scales, Amersham), and exposed for 8 weeks at room temperature.

Films were developed for 3 min in Kodak D19 developer, shortly rinsed in tap water, fixed for 5 min in 30% sodium thiosulphate solution, rinsed for 5 min in running tap water, and dried. For quantification, an automatic image analysis system (Leica, Quantimet) was used. The optical
density in several brain areas was measured in two sections per rat and [3H] 8-OH-DPAT binding was calculated.

2.3. The 5-HT$_{1A}$ challenge test

The hypothermic response after administration of the selective 5-HT$_{1A}$ agonist alnespirone [3,9,14,31] was studied. All injections were given between the first and fourth hour of the dark phase. In rats, a radio-telemetry system (Data Sciences, St. Paul, MN) was used for stress-free monitoring of body temperature. Aggressive ($n=13$) and nonaggressive ($n=11$) rats were housed individually in Plexiglas cages (40 $\times$ 23 $\times$ 15 cm). Transmitters (TA10TA-F40; sensitivity: 0.1°C) were implanted intraperitoneally under general halothane anesthesia. Rats were allowed to recover for at least 10 days, and by that time, they had regained a stable circadian rhythm in body temperature and activity. Subcutaneous (sc) injections of alnespirone (20 mg/kg) or vehicle (distilled water) were given within 4 min to all rats after which they were left undisturbed for measurement of the body temperature response. Every animal was injected with vehicle and alnespirone in a random order, with 1 week between consecutive injections. Temperature was measured every 5 min.

In mice, body temperature was measured using a rectal probe (sensitivity: 0.1°C), which was inserted for 1.5 cm. The temperature response to alnespirone (20 mg/kg sc) or vehicle (distilled water, sc) was determined, measuring body temperature before and 20 min after injection. To test the site of action, the body temperature response to S-15535 (20 mg/kg sc) administration and to alnespirone administration with S-15535 pretreatment (15 min) were also measured. Because there was no qualitative difference between the data of aggressive and nonaggressive mice, the results were combined to facilitate the interpretation. Each group consisted of an equal number of aggressive and nonaggressive mice.

2.4. Drugs

Alnespirone [(+)-S-20499-2 hydrochloride; lot no. 48647, molecular weight: 479] and S-15535-3 methanesulfonate [4-(benzodioxan-5-yl)-1-(indan-2-yl)piperazine; lot no. EI798, molecular weight: 432.5] were provided by the Institut de Recherches Internationales Servier, France. Both drugs were dissolved in sterile distilled water (vehicle solution) and injections were given subcutaneously, in rats in the flank in a volume of 1 ml/kg body weight, and in mice in the neck in a volume of 0.1 ml/20 g. Vehicle and solutions were at room temperature when injected.

2.5. Statistical analysis

A Student’s $t$ test was used to analyse differences between aggressive and nonaggressive individuals, with regard to [3H] 8-OH-DPAT binding in rats and temperature responses in mice. The temperature responses in rats were analysed using an ANOVA for repeated measurements, and post hoc analysis at time points was done with a Student’s $t$ test. In all cases, differences were considered significant if $P<.05$.

3. Results

3.1. 5-HT$_{1A}$ receptor binding

Binding of [3H] 8-OH-DPAT in a number of brain areas in rats is shown in Fig. 1. There was no difference in binding between aggressive and nonaggressive rats in any of the brain areas measured.

3.2. Hypothermic response

In rats (Fig. 2A), administration of alnespirone induced a hypothermic response, while the vehicle injection caused a short-lasting increase in body temperature. The overall analysis of the responses with ANOVA for repeated measurements ($r=0$ till 240 min), with variables time, group, and treatment, resulted in a significant effect of time [$F(48,2112)=39.439; P<.001$] and treatment [$F(1,44)=72.138; P<.001$] and a time $\times$ treatment interaction [$F(48,2112)=21.058; P<.001$]. There were also significant interactions of time $\times$ group [$F(48,2112)=1.656; P<.01$] and time $\times$ group $\times$ treatment [$F(48,2112)=1.742; P<.01$], indicating a different response pattern in aggressive versus nonaggressive individuals. Analysis of the treatments separately resulted for the alnespirone treatment in a significant time effect [$F(48,1056)=52.719; P<.001$] and a significant time $\times$ group interaction [$F(48,1056)=1.464; P<.05$]; for the vehicle treatment, there was also a significant time effect [$F(48,1056)=9.942; P<.001$] and a significant time $\times$ group interaction [$F(48,1056)=1.911; P<.001$]. The differences within individuals between the responses to alnespirone and vehicle

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**Fig. 1.** [3H] 8-OH-DPAT binding in several brain areas of aggressive ($n=6$) and nonaggressive ($n=5$) rats (mean $\pm$ S.E.M.).
were calculated (Fig. 2B) and analysed with ANOVA for repeated measurements, giving a significant effect of time [\(F(48,1056) = 21.188; P < .001\)] and interaction of time \(\times\) group [\(F(48,1056) = 1.752; P < .01\)]. Post hoc analysis with a one-sided \(t\) test for independent samples gave between \(t = 35\) and 185 min difference between groups at 17 out of the 31 time points with a \(P\) value < .05, and another 9 with \(0.05 < P < .10\). The baseline period (Fig. 2A: \(t = 60\) till \(-5\) min) was analysed separately, resulting in a significant treatment effect [\(F(1,44) = 6.055; P < .05\)] and a time \(\times\) treatment interaction [\(F(11,484) = 2.291; P < .05\)]. When analysing the treatments separately only within alnespirone treatment, a significant effect was found for time [\(F(11,242) = 3.591; P < .001\)]. During the baseline period, there were no differences between groups.

The hypothermic response after alnespirone administration in mice (Fig. 3) was also strongest in the aggressive individuals (two-sided \(t\) test for independent samples: \(P < .05\)), while the response to the vehicle injection did not differ between groups.

Fig. 4 shows that alnespirone caused a hypothermic response in mice. S-15535 administration had no effect on body temperature by itself, but it blocked the hypothermic effect of alnespirone. Analysis of the data with a two-sided \(t\) test for paired samples showed that the response to alnespirone differed significantly from vehicle \((P < .001)\) and from S-15535 \((P < .001)\) treatment, while the response to alnespirone with S-15535 pretreatment differed significantly from the ones to vehicle \((P < .01)\), to alnespirone alone \((P < .01)\), and to S-15535 alone \((P < .01)\).

**4. Discussion**

The main results of the studies presented in this paper are: first, no difference was found between aggressive and non-aggressive rats in 5-HT1A receptor binding in any of the brain areas measured; and second, the hypothermic response to a
challenge with the 5-HT_{1A} receptor agonist alnespirone was enhanced in aggressive individuals, both in rats and mice.

When Korte et al. [16] studied 5-HT_{1A} receptors in mice, they found that [3H] 8-OH-DPAT binding and mRNA expression in a number of postsynaptic areas were enhanced in individuals from the aggressive selection line. In contrast with these findings, no difference in binding could be shown between aggressive and nonaggressive rats in the present experiment. But when studying the functionality of the receptor system, a response mediated via postsynaptic 5-HT_{1A} receptors was stronger in aggressive rats and mice than in nonaggressive ones.

It can be concluded from these results that postsynaptic 5-HT_{1A} receptors are more sensitive in aggressive individuals. It is surprising, however, that in rats, only in the functional response a difference was found, while the mice also differed in receptor binding and mRNA expression. This may be explained by the different backgrounds of the two groups of experimental animals: Although both in rats and mice aggressive and nonaggressive individuals are studied, an important distinction is that the rats are selected individually from a randomly bred strain, while the mice came from genetic selection lines. Differences in behaviour and physiology seem to be much more pronounced between the mouse lines than between individual rats. A functional response is the result of many different elements, like the number of receptors and binding of the drug to the receptors, but also a cascade of intracellular processes. Small differences at various levels in the signal transduction pathway may finally give a different functional response, while at none of the separate levels (e.g., receptor binding) can a difference be shown. Functionality of a receptor system and differentiation herein are biologically relevant phenomena. That in the mice already significant changes were found at the receptor level (in the 'hardware') may be specific for selection lines.

A few aspects of the Results section still need additional explanation. First, there were a significant time effect and a time × group interaction in the temperature response of rats to vehicle administration (Fig. 2A). After the hyperthermic response, caused by handling and injecting the rats, body temperature returned to basal values. Under basal conditions, there are always minor variations in body temperature, which are not necessarily equal for all groups. The difference in time course between aggressive and nonaggressive rats may be caused by variation in basal values and/or a slightly stronger hyperthermia after injection in aggressive individuals. Anyway, the difference in response to alnespirone cannot be explained by the difference in response to vehicle, as is shown in Fig. 2B. Likewise, the treatment effect and the time × treatment interaction during the baseline period are probably due to normal variations in body temperature under basal conditions, as described above. Again, they are not explaining the hypothermic effect of alnespirone, but rather enhancing this effect.

Although the exact mechanism of action is unknown, there is strong evidence that the hypothermic response is mediated via central postsynaptic 5-HT_{1A} receptors. Therefore, this response is often used as a model to show agonistic action of a drug on postsynaptic 5-HT_{1A} receptors, at least in rats [1,18,21,27,36]. As far as mice are concerned, the literature is contradictory. In this species, the hypothermic response is thought to be mediated via 5-HT_{1A} autoreceptors [2,10,11,19]. A complicating factor in extrapolating data to other species is that pharmacological effects may have differential agonistic or antagonistic effects in distinct species, or they may differentially affect other receptor systems as well. As an example, Moser [26] showed that in mice, the α1 adrenoceptor is involved in the hypothermic response to 8-OH-DPAT to a greater extent than it is in rats. Furthermore, NAN-190, which is regarded to be a 5-HT_{1A} receptor antagonist, has a clear antagonistic action in rats, but not in mice. These differential pharmacological effects make it difficult to understand the mechanism and may have contributed to the contradictory interpretation of data.

In the experiments described here, alnespirone, a selective 5-HT_{1A} receptor agonist [3,9,14,31], was administered. In contrast to several other drugs acting at the 5-HT_{1A} receptor [21,26], no agonistic action of alnespirone on adrenergic receptors has been reported. So, effects of this drug on body temperature mediated via other receptor systems are unlikely. To be able to discriminate between an action mediated via autoreceptors and postsynaptic receptors, S-15535 was used, which acts as an agonist at the 5-HT_{1A} autoreceptor and has antagonistic properties on the 5-HT_{1A} postsynaptic receptor [22–24,30]. In mice (this experiment) as well as in rats [8,22,23,30], this drug does not induce hypothermia by itself, but antagonises the effect of alnespirone or 8-OH-DPAT. From these data we may conclude that the hypothermic response is mediated via postsynaptic 5-HT_{1A} receptors, both in rats and mice. And as such, it is legitimate to use this response as a measure of postsynaptic 5-HT_{1A} receptor function in both species.

A number of clinical studies investigating 5-HT_{1A} receptor function and aggression found a negative correlation (or no correlation) between certain measures of aggression and thermal responses to 5-HT_{1A} challenges [4,7,25]. This is contradictory to the results in this paper. A factor to take into account when interpreting these data is that in most of these experiments (part of), the human subjects are patients, who have used medication, acting amongst others at the serotonergic system. It is inherent to this treatment that changes in 5-HT receptor sensitivity are introduced; what is more in most cases, the therapeutic effect is dependent on changes in the 5-HT system. This may seriously interfere with the correlations found between aggression and receptor sensitivity, measured by a functional response. The direction of the correlation may very well be a result of treatment, and not of the original trait characteristics of the subject. In this study, the animals have not been treated before, and the
differences found are more likely to have a direct relation with the level of aggressiveness of the animal itself.

To conclude, the results of these experiments show an increased sensitivity of postsynaptic 5-HT1A receptors in individuals with a high trait aggression, compared to those with a low trait aggression. This difference is more pronounced between the mouse selection lines than it is within the randomly bred rat strain: While in the former it was found in the number of receptors and in functionality, in the latter, it could only be seen in receptor function. The enhanced sensitivity of the postsynaptic part of the 5-HT system in aggressive individuals could be due to a lower basal serotonergic neurotransmission in those individuals, supporting the 5-HT deficiency hypothesis of aggression.

Acknowledgments

We thank Jan Keijser for his assistance in the autoradiographic study.

References


