Serotonin, cortisol, and stress-related psychopathology

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Chapter 3

Opposite effects of a 5-HT_{1A} and a 5-HT_{2C} receptor agonist on the acoustic startle in rats on a low tryptophan diet, preliminary results

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ABSTRACT

Objective: *In several somatic diseases and treatment conditions, low tryptophan plasma levels are associated with increased aggression and irritability rather than depression. Previously, we have used a low tryptophan diet to mimic this condition in rats and found an increase of the acoustic startle response only when immobilization stress was also applied. In the present study we investigated the potential of four selective 5-HT receptor agonists to modify the previously noted stress induced increase of the acoustic startle in rats on a low tryptophan diet.*

Methods: *Acoustic startle response, habituation and pre-pulse inhibition were measured in five groups of rats on a low tryptophan diet and in one group on a normal diet. Agonist effects were measured in the low tryptophan condition only, while the effects of saline were measured in both conditions. Saline and agonist injections (s.c.) took place 30 min prior to behavioral testing. This procedure was repeated on the consecutive four days. Doses of the agonists were based on behavioral studies from literature.*

Results: *A low tryptophan diet itself did not affect the response and habituation to the acoustic startle, nor did it influence prepulse inhibition. However, significant increases and decreases in startle response were observed in combination with the 5-HT_{1A} agonist flesinoxan and the 5-HT_{2C} agonist Ro600175, respectively. The effects of both agonists on the acoustic startle were comparable within the five days test period, indicating that rapid desensitization of the receptors involved does not occur.*

Conclusion: *Administration of a 5-HT_{2C} receptor agonist has the potential to ameliorate the increased irritability and hostility in patients with low circulating tryptophan levels. Likewise, co-administration of a 5-HT_{1A} receptor antagonist with an antidepressant might decrease the irritability and anxiety associated with the early phase of treatment.*

INTRODUCTION

Serotonergic pathways have been implicated in mood and a variety of behaviors (54;55). Synthesis of serotonin (5-HT) in the central nervous system (CNS) is highly dependent on the plasma levels of its precursor molecule tryptophan (TRP), which is an essential amino acid. Consequently, central 5-HT function may be manipulated by dietary TRP (47;180;181).

In humans, low plasma TRP levels have been associated with decreased mood, and more recently also with hostility as well as impaired impulse control (63;66;67;116;133). Similarly, low serotonergic activity has been associated with increased impulsivity and aggression in rodents (69;70;152).

Such observations might connect with an increased responsiveness to stressors. Divergent measures for stress sensitivity have been applied in animal research, but only few of them have a pendant in human research. An example of the latter is the acoustic startle reflex (ASR) which can be elicited in rats and humans essentially by using identical stimulus parameters to generate response patterns (126). Indeed, startle reactivity is enhanced by 5-HT depletion either through electrolytic lesions of the dorsal and median raphe nuclei (164) or pharmacologically by inhibition of 5-HT synthesis (71;153), but also by a TRP-free diet (73). Recently, we have shown that a low TRP diet increased stress-sensitivity as measured through the response to acoustic stimuli of rats that were also subjected to immobilization stress (182).

A change of 5-HT function may also influence prepulse inhibition (PPI), the phenomenon that a startle response to a strong stimulus is reduced when it is preceded by a low intensity stimulus within a short time span (123). Examples are the disruption of PPI by pharmacologically increasing central 5-HT release in rats (155) and the attenuation of PPI, without altering the stronger basal startle response, following 5-HT depletion with the 5-HT synthesis inhibitor *p*-chlorophenylalanine (156;183). The relationship between cerebral 5-HT functioning and ASR has been investigated extensively. However, the receptor mechanisms involved have received lesser attention.

Until now, 14 structurally and pharmacologically distinct mammalian 5-HT receptor subtypes have been identified, which are now assigned to one of seven families 5-HT$_{1–7}$ (51). The 5-HT$_{1A}$ receptor and 5-HT$_{1B}$ receptors are auto-receptors which modulate the neuronal release of 5-HT (52;53;184). 5-HT$_{1A}$ receptor agonists have been shown to enhance the startle response (156). The other 5-HT receptors are predominantly located postsynaptically. When it concerns the attenuation of the startle reflex and more specifically the PPI, several 5-HT receptor subtypes may be involved. For instance, selective stimulation of 5-HT$_{2A}$ (185;186), 5-HT$_{1B}$ (187;188), and 5-HT$_{1A}$ receptors (186;189) has been reported to disrupt PPI in rats, albeit not unequivocally (190).

In the present pilot study we have investigated the potential of specific 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} and 5-HT_3 receptor agonists to modify (stress) reactivity in rats with low circulating levels of TRP. Doses of the agonists were based on behavioral studies from literature (191-196).

Low circulating tryptophan levels were achieved by dietary TRP depletion (47). Reactivity was measured by the response and habituation to acoustic startle pulses (76;123;125).

MATERIALS AND METHODS

Animals and overall design

The experimental procedures were approved by the Animal Ethics Committee of the University of Groningen. Every reasonable effort was made to minimize the animals' discomfort and the number of animals used.

Thirty-six male Wistar rats (Harlan, the Netherlands) weighing 323 ± 2 g at the start of the experiment were housed individually in plexiglas cages (45 cm x 28 cm x 20 cm) in a temperature controlled environment (21-23 °C). Animals were kept on a 12h reversed light/dark cycle with lights on from 1900h to 0700h. The experiments were conducted in the dark (active) period. All animals were handled daily to minimize handling stress during the experiment. Food intake and body weight were measured daily. The animals had ad libitum access to water and food.

At day –4 the standard rat chow was replaced by a synthetic control diet. At day 0, after 10 days of acclimatization, animals were assigned to one of two treatment groups: 30 animals were assigned to a synthetic low TRP diet (Trp^-), 6 animals to a synthetic control diet (Trp^+). At day 3 startle experiments started. The Trp^- animals were divided into five treatment groups, each consisting of 6 rats.

The animals were subjected to daily acoustic stimulus sessions during five days. At day 7 rats were anesthetized using isoflurane anesthesia and then decapitated. Thymus and adrenals were removed and weighed.

Diet

All diets were designed by Numico, Wageningen, the Netherlands and manufactured by Research Diet Services B.V., Wijk bij Duurstede, the Netherlands. The Trp^+ diet contained 0.24 g Trp / 100 g diet, the Trp^- diet contained only 0.024 g TRP/100 g diet, as described by van der Stelt et al. (162). To equalize the total amount of amino acid in the diets changes in Trp content were counterbalanced by adjusting the amounts of leucine, isoleucine and valine (LNAA's).

Administration of 5-HT receptor agonists

Four different 5HT agonists were tested (table 1). Control animals (6 Trp^- and 6 Trp^+) were injected with saline.

All 5-HT receptor agonists were dissolved in saline, if necessary with the aid of a temperature controlled ultrasonic water bath. The agonists were injected subcutaneously (1ml/kg) 30 min prior to the startle procedure, allowing intrinsic locomotor effects to abate.

Table 1: Treatment groups and doses

Group	Diet	Agonist	Treatment	Dose (mg/kg)
Control	Trp+	None	Saline	1
Low TRP	Trp-	None	Saline	1
$5\text{-}HT_{1A}$	Trp-	$5\text{-}HT_{1A}$	Flesinoxan	3
$5\text{-}HT_{2A}$	Trp-	$5\text{-}HT_{2A}$	DOI	0.25
$5\text{-}HT_{2C}$	Trp-	$5\text{-}HT_{2C}$	Ro600175	0.25
$5\text{-}HT_3$	Trp-	$5\text{-}HT_3$	m-chlorophenylbiguanide	5

Startle procedures

The individual rats were tested at the same time daily. The behavioral experiments were carried out in a separate room. Startle experiments were performed using a one-unit automated TSE startle response system (TSE Systems, Bad Homburg, Germany). Animals were tested in small plexiglas cages (100 x 60 x 90 mm) restricting major movements and exploratory behavior. The cage featured an integrated stainless steel floor grid and a feces tray. The cage was placed on a highly sensitive transducer platform. During the sessions, the animals remained in the cages within a sound-attenuating cabinet. Here a 70dB white background noise and auditory stimuli were generated by means of speakers mounted into this cabinet. Stimuli were delivered and startle responses measured by the TSE software running on a PC next to the startle cabinet.

The rats were subjected to the same conditional program every day. The startle procedure proceeded as follows. A 150 s acclimatization period, during which the rats received no stimuli, was followed by a 150 s baseline period, during which the rats received no stimuli except for a 70dB background noise. The test session began with 10 trials consisting of single 40 ms 120 dB white-noise startle stimuli. The sessions then continued with 50 trials consisting of random delivery of twenty 120 dB pulse-alone trials, twenty prepulse trials and 10 trials during which no stimuli were delivered. Prepulse trials included a single 120 dB pulse 100 ms preceded by a 20 ms prepulse-stimulus of 80 dB. Percentage of PPI was calculated as [(startle response with the prepulse – response to the middle twenty 120 dB pulses)/response to the middle twenty 120 dB pulses x 100]. The last ten trials were single

40ms 120dB pulse-alone startle stimuli. The total of 70 trials was delivered with a constant interval of 10s. The first and last 10 pulse-alone stimuli (block 1 and block 3, respectively) and the 20 pulse-alone stimuli included in the PPI block itself (block 2) were used to obtain a measure of response habituation in response to repeated delivery of startling stimuli. Startle reaction was measured as the maximum response (startle amplitude) to a stimulus.

Statistical analyses

Statistical analyses were done using SPSS (version 12.0), with significance determined at $p \leq 0.05$. Data are shown as mean ± S.E.M. Organ weight (corrected for body weight) was analyzed using a one-way ANOVA. Weight gain, ASR and PPI were analyzed using a repeated measurement ANOVA with treatment and group as between subject factors and day (1-5) and block (1-3) as within subject factors. Greenhouse-Geisser correlations were used when the assumption of sphericity was not met. Correlations were counted using the Pearson correlation coefficient.

RESULTS

Body weight, organ weight and food intake

Before implementing the experimental diet conditions body weight did not differ between the treatment groups (323 ± 2 g). Thereafter a main effect of day was found ($F(6,25) = 14.735$; $p < 0.001$) as well as a day*group interaction effect ($F(3,102) = 3.519$; $p < 0.001$), whereas the TRP normal animals continued to grow, the other animals didn't grow or even lost some weight. The Post Hoc tests revealed a significant effect of diet ($p < 0.001$). For food intake a main effect of day was found ($F(6,25) = 36.996$, $p < 0.001$) as well as a main effect of group ($F(5,30) = 11.195$, $p < 0.001$) and an interaction effect of day and group ($F(30,102) = 2.172$, $p = 0.001$). Post Hoc tests revealed a significant effect of the TRP low diet as compared to the TRP normal controls. Agonists had no effect on food intake. The TRP low diet had no effects on organ weight.

Behavioral effects of the 5-HT receptor agonists

The 5-HT_{1A} receptor agonist flesinoxan elicited several symptoms of the serotonin behavioral syndrome including flat body posture, lower lip retraction and hind limb abduction. The 5-HT_{2C} receptor agonist Ro600175 induced a short-lasting suppression of motor functioning, as reported previously (197). However, in all cases the effects of flesinoxan and Ro600175 had faded before the startle experiments commenced. The other agonists were without overt behavioral effects.

Startle response

Repeated exposure to the acoustic stimuli resulted in a decrease in motor response in all animals within one session (short-term habituation; main effect of block $F(2,29) = 104.613$, $P < 0.001$), but not in all animals over the days (long-term habituation; main effect of day $F(4,27) = 1.256$, $P = 0.31$) (fig. 1). Difference in habituation between both treatment groups was seen by a day x group interaction effect ($F(5,30) = 2.799$, $p = 0.034$) and an interaction effect of block x group ($F(10,58) = 3.117$, $P = 0.003$). Post Hoc Bonferroni tests revealed no effect of the low TRP diet on habituation. A significant difference on habituation was found between low TRP controls and both the 5-HT_{1A} ($p = 0.003$) and 5-HT_{2C} ($p = 0.008$) agonist treated animals.

Significant between group-effects were also found between low TRP controls and groups treated with 5-HT_{1A} ($p < 0.001$) and 5-HT_{2C} ($p < 0.001$) agonists. Treatment did not affect prepulse inhibition (PPI). There was however, an effect of day on PPI ($F(4,27) = 4,127$, $p = 0.01$)

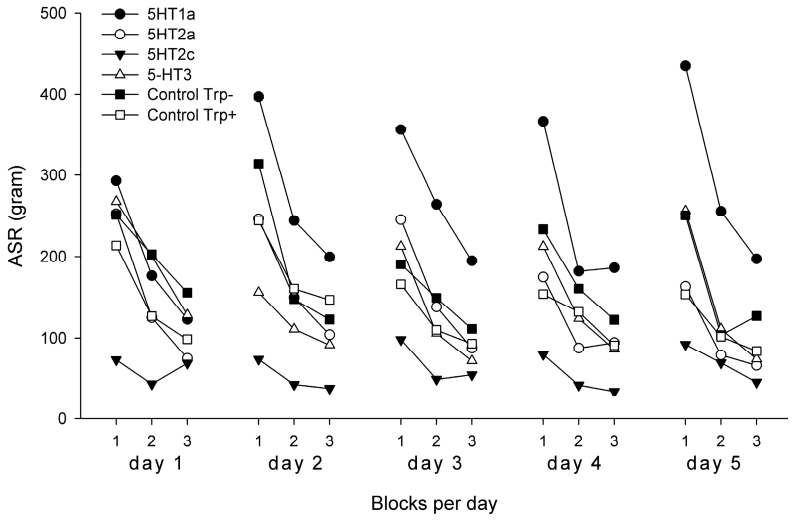

Figure 1: Effects of specific 5-HT receptor agonists on startle responses during the 5 test days, subdivided in 3 blocks per daily session. Agonists were administered to low tryptophan rats only. Closed circle: 3 mg/kg flesinoxan (s.c.); open circle: 0.25 mg/kg DOI (s.c.); closed reversed triangle: 0.25 mg/kg Ro600175 (s.c.); open triangle: 5 mg/kg m-chlorophenylbiguanide (s.c.); closed square: saline (s.c.) + low tryptophan diet; open square: saline (s.c.) + normal tryptophan diet.

DISCUSSION

A low TRP diet alone did not alter the acoustic startle response, but the ASR was significantly increased in combination with a 5-HT_{1A} receptor agonist and decreased in combination with a 5-HT_{2C} receptor agonist.

Previously we observed an increased response to acoustic stimuli in rats on a low TRP diet only when immobilization stress was also applied (182). This is in agreement with a study by Yunger et al. (175), reporting that lesions of the medial forebrain bundle did not alter the magnitude of noise-elicited startle unless foot-shocks were applied prior to the test session. Another confounding factor could be that food deprivation itself may depress the startle response (174). Walters et al. reported that a low TRP diet led to significant changes in startle behavior only when rats were nourished adequately (73). In the present study, rats on a TRP low diet ate significantly less than the control animals, which is in agreement with the reported role of serotonin in satiety (198). However, to which extent the decreased food intake itself has interfered with our study is difficult to judge. The low TRP diet did not alter PPI, which is in contrast with previous studies that depleted 5-HT by means of 5,7-dihydroxytryptamine or p-chlorophenylalanine (155;156). We have no clear-cut explanation for the lack of effect on PPI in our study other than a low TRP diet being less inflicting than the pharmacological interventions. On the other hand, the TRP low diet of three days as applied here has proven to decrease the brain content of 5-HT considerably (47), which might indicate that other factors are at play.

The 5-HT_{1A} receptor agonist flesinoxan significantly enhanced startle reactivity, while the 5-HT_{2C} receptor agonist (Ro600175) effectively decreased it. Flesinoxan decreases neuronal release of serotonin through activation of 5-HT_{1A} autoreceptors in the raphe nuclei (184). It is conceivable that this pharmacological effect adds to the already low levels of serotonin achieved through the low TRP diet. This idea is consistent with previous studies, wherein 5-HT depletion and a 5-HT_{1A} receptor agonist had a comparable effect on the ASR (156;199). Arguably, this makes an involvement of postsynaptic 5-HT_{1A} receptors in the ASR less likely.

The selective agonists for 5-HT_{2A} and 5-HT_3 receptors had no effect on PPI or startle amplitude, which corroborates previous studies regarding their involvement in the ASR (200;201). Because we used only one dose of each agonist it is, however, difficult to draw definitive conclusions in this respect.

The observation that the 5-HT_{2C} agonist Ro600175 significantly decreased startle reactivity may be of clinical interest. Importantly no signs of tolerance to this effect were noted during the 5 days test period. This indicates that rapid desensitization of the 5-HT_{2C} receptors involved does not occur. The selective 5-HT_{2C} receptor agonist Lorcaserin (202) is currently subject of clinical trials for its alleged anti-obesity properties, but it might also ameliorate some of the the psychiatric side-effects associated with clinical conditions (e.g. carcinoid tumor, inflammation) and medications (e.g. interferon-α with hepatitis C and various

cancers), where aggression and failing impulse control are associated with low circulating TRP plasma levels (66;67;115;116). Finally, because selective serotonin reuptake inhibitors (SSRIs) act as indirect agonists of both 5-HT_{1A} and 5-HT_{2C} receptors, it can be speculated that co-administration of a 5-HT_{1A} receptor antagonist with an SSRI might decrease the irritability and anxiety associated with the early phase of treatment (see also (203)).

Conclusion

A low TRP plasma level influences the ASR in rats only in combination with a 5-HT_{1A} or a 5-HT_{2C} receptor agonist. On the basis of the present data and our previous study, wherein low TRP plasma levels were associated with increased stress sensitivity, it can be speculated that administration of a 5-HT_{2C} receptor agonist might ameliorate the increased irritability and hostility in patients with low circulating TRP levels. Alternatively, co-administration of a 5-HT_{1A} receptor antagonist with an SSRI might decrease the irritability and anxiety associated with the early phase of treatment.

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