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Partial and Complete Blockade of 5-Hydroxytrytophan (5-HTP)-Induced Head Twitches in the Rat: A Study of Ritanserin (R 55 667), Risperidone (R 64 766), and Related Compounds

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ABSTRACT

Meert T.F., C.J.E. Niemegeers, F. Awouters, and P.A.J. Janssen: Partial 5-hydroxytryptophan and complete blockade of (5-HTP)-induced head twitches in the rat. A study of ritanserin (R 55 667), risperidone (R 64 766) and related compounds. Drug Dev. Res. **13**:237–244, 1988

A series of test compounds were studied for their ability to inhibit and block the head-twitch response to either intraperitoneal (i.p.) 5-hydroxytryptophan (5-HTP) or intravenous (i.v.) mescaline in rats. Both responses were found to be sensitive to serotonin S_2 antagonists, and there was very good agreement between the inhibitory doses in both tests, particularly for the selective serotonin S_2 antagonists ritanserin and seganserin. However, these two compounds did not block the 5-HTP response, although they completely abolished the mescaline response. In contrast, the mixed serotonin-dopamine-norepinephrine antagonist risperidone was a potent blocker of both responses. The use of various antagonists and the combination treatments of ritanserin with haloperidol or prazosin indicated that the 5-HTP response is abolished when potent serotonin S_2 antagonism is associated with antagonistic activity on either dopamine D_2 or α_1 receptors.

Key words: 5-HTP head twitches, mescaline head twitches, serotonin S_2, catecholamines, ritanserin, risperidone

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INTRODUCTION

Head-twitch responses in rats after injection of 5-hydroxytryptophan (5-HTP) or mescaline have been related to central stimulation of serotonin receptors, mainly on the basis of dose-dependent reduction of the number of head-twitches by compounds that block these receptors [Colpaert and Janssen, 1983; Corne et al., 1963; Goodwin and Green, 1985; Green et al., 1983; Matthews and Smith, 1980; Martin et al., 1985; Niemegeers et al., 1983; Peroutka et al., 1981].

The fact that 5-HTP is a direct precursor of serotonin suggests that this amine may be the only mediator of the head-twitch response. Several studies on 5-HTP, however, have drawn attention to a concomitant increase in dopamine and norepinephrine turnover [Awazi and Goldberg, 1978; Everett, 1979; Van Praag, 1983]. The potential involvement of catecholamines in head-twitch responses now can be studied with an enlarged series of antagonists. In addition to well-known dopamine and norepinephrine antagonists, selective serotonin S_2 antagonists (ritanserin and seganserin) and various compounds with mixed serotonin-dopamine-norepinephrine antagonism (such as risperidone) were used. Provisional conclusions were tested further by combination treatments with selective neurotransmitter antagonists.

MATERIALS AND METHODS

Subjects

The subjects were male Wistar rats of body weight 240 ± 40 g, bred in the animal quarters of Janssen Pharmaceutica. The rats were food deprived overnight, with tap water freely available except during testing. For observation, the rats were placed in perspex cages with a wire-mesh floor (13 × 20 × 16 cm). The experiments were carried out in air conditioned laboratory rooms (temp. 21 ± 1°C; R.H. 65 ± 15%). Rats were used only once.

Test Procedures

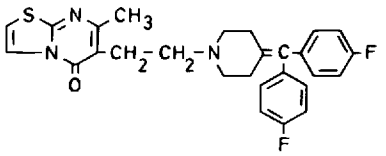
The 5-HTP test was performed as described previously [Colpaert and Janssen, 1983]. Head-twitches (TWI) were induced by intraperitoneal injection of 320 mg/kg (2 ml/100 g body weight (b.w.)) of 5-HTP and counted over a 20-min period, starting 70 min after injection.

The mescaline (MES) test also has been described previously in detail [Niemegeers et al., 1983]. TWI were induced by intravenous (i.v.) injection of 20 mg/kg (0.2 ml/100 g b.w.) of MES and counted over a 15-min period following the injection.

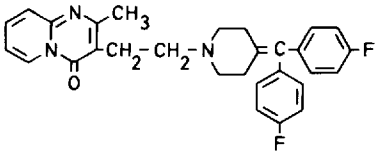
In the apomorphine-tryptamine-norepinephrine (ATN) test [Niemegeers et al., 1977], rats were injected i.v. with 1.25 mg/kg apomorphine (t = 0), followed by 40.0 mg/kg tryptamine (t = + 60 min) and 1.25 mg/kg norepinephrine (t = + 90 min). Agitation and stereotypies in the apomorphine test (60-min observation period), bilateral clonic seizures of the forepaws, and coarse body tremors in the tryptamine test (5-min observation period), and lethality in the norepinephrine test within 120 min after injection were evaluated.

Compounds

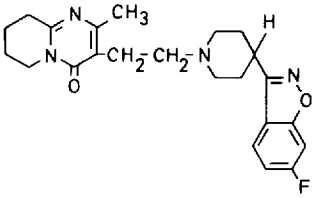
The compounds under study were injected subcutaneously (s.c.) (1 m/100 g b.w.) 1/2 hr (ATN), 2 hr (5-HTP), and 1, 2, 4, and 8 hr (5-HTP and MES with ritanserin, seganserin, and risperidone) before testing. Aqueous solutions used included: ketanserin tartrate and seganserin (R 56 413); butanserin (R 53 393) and tioperidone HCl using 1 equivalent (eq.) tartartic acid (H_2T); of haloperidol, pirenperone (R 47 465), pimozide, risperidone (R 64 766), ritanserin (R 55 667) using 2 eq. H_2T; and prazosin HCl, using 3 eq. H_2T. The doses were selected from the geometric series 0.00125, 0.0025, . . . , 5.00, 10.0 mg/kg. For each dose level, five rats were used.



RITANSERIN (R55667)



SEGANSERIN (R56413)



RISPERIDONE (R64766)

Fig. 1. Chemical structures of ritanserin, seganserin, and risperidone.

Statistics

ED_{50} values and confidence limits were calculated as mg/kg base of the compounds using Finney's interative method [Finney, 1962], according to an "all or none" criteria derived from the analysis of the control results obtained in this and previous studies.

RESULTS

The median number of TWI's induced by 320 mg/kg i.p. 5-HTP in a series of 166 saline pretreated controls was 52, ranging from 0 to 177. Five rats (3.0 %) had < 10, and one rat (0.6 %) < 2 TWI. The median number of TWI induced by 20 mg/kg i.v. of MES in a series of more than 800 controls was 24, ranging from 0 to 102. Fewer than 5 TWI occurred in 2.6% and < 2 TWI in 0.6 % of the control population. No TWI were observed in rats injected i.p. or i.v. with saline instead of 5-HTP (n = 70) or MES (n = 40), respectively. Based on these data, inhibition of TWI was defined as < 10 (5-HTP) and < 5 (MES), whereas for blockade < 2 was used in both tests.

The results obtained with ritanserin, seganserin, and risperidone at different intervals with respect to the injection of 5-HTP or MES are shown in Table 1. The three compounds inhibited the 5-HTP-induced TWI. Peak effect with ritanserin (ED_{50}: 0.074 mg/kg) and seganserin (0.19 mg/kg) was reached at 2 hr and reached with risperidone at the 1-hr interval (ED_{50}: 0.016 mg/kg). Blockade of 5-HTP-induced TWI was obtained with risperidone (ED_{50}: 0.056 mg/kg), but not with ritanserin or seganserin at doses up to 10.0 mg/kg. In the MES test, inhibition and—at slightly higher doses—blockade of TWI were obtained with the three compounds. Comparing for each compound the ED_{50}'s that inhibit 5-HTP- and MES-induced

TABLE 1. ED_{50}'s and Confidence Limits (in mg/kg) Obtained With Ritanserin, Seganserin, and Risperidone at Stated Intervals in the 5-H and MES Tests in Rats

Compounds	Pretreatment hrs	Head Twitches			
		5-HTP-induced		MES-induced	
		Inhibition	Blockade	Inhibition	Blockade
Ritanserin	1	0.39 (0.22–0.66)	>10.0	0.13 (0.075–0.22)	0.13 (0.075–0.22)
	2	0.074 (0.043–0.13)	>10.0	0.085 (0.053–0.14)	0.13 (0.080–0.21)
	4	0.15 (0.077–0.28)	>10.0	0.15 (0.088–0.25)	0.34 (0.21–0.54)
	8	0.51 (0.27–0.98)	>10.0	0.44 (0.26–0.76)	0.89 (0.59–1.33)
Seganserin	1	0.51 (0.30–0.88)	>10.0	0.39 (0.28–0.58)	0.58 (0.39–0.88)
	2	0.19 (0.12–0.31)	>10.0	0.22 (0.15–0.33)	0.34 (0.21–0.55)
	4	0.34 (0.20–0.58)	>10.0	0.39 (0.26–0.58)	1.02 (0.59–1.75)
	8	2.35 (1.30–4.27)	>10.0	1.34 (0.83–2.17)	1.77 (1.10–2.86)
Risperidone	1	0.016 (0.012–0.022)	0.056 (0.033–0.096)	0.037 (0.027–0.050)	0.049 (0.036–0.066)
	2	0.021 (0.013–0.034)	0.097 (0.065–0.15)	0.064 (0.043–0.096)	0.097 (0.057–0.17)
	4	0.11 (0.069–0.18)	0.44 (0.28–0.72)	0.29 (0.17–0.51)	0.45 (0.28–0.72)
	8	2.35 (1.29–4.26)	9.36 (6.25–14.11)	Not tested.	Not tested.

TABLE 2. ED_{50}'s and Confidence Limits (in mg/kg) Obtained With Different Compounds and Combinations of Compounds in the 5-HTP Antagonism Test in Rats*

Compounds	5-HTP-induced TWI	
	Inhibition	Blockade
Pirenperone	0.012 (0.0061–0.025)	0.049 (0.027–0.089)
Ketanserin	0.26 (0.14–0.47)	2.70 (1.49–4.90)
Butanserin	0.51 (0.28–0.93)	1.78 (1.19–2.66)
Haloperidol	0.67 (0.39–1.16)	1.34 (0.99–1.82)
Pimozide	>10.0	>10.0
Prazosin	>10.0	>10.0
Tioperidone	>10.0	>10.0
Haloperidol + prazosin 10.0 mg/kg	>0.31	>0.31
Ritanserin	0.074 (0.043–0.13)	>10.0
Ritanserin + haloperidol 0.08 mg/kg[a]	0.049 (0.028–0.084)	0.19 (0.10–0.37)
Ritanserin + prazosin 0.04 mg/kg[a]	0.056 (0.033–0.096)	0.29 (0.18–0.47)

*All injections were given s.c. at 120 min before testing.
[a]The median number of TWI after pretreatment with haloperidol 0.08 mg/kg alone was 22 (13–64) and with prazosin 0.04 mg/kg alone 47 (18–61).

TWI, virtually no differences were observed for ritanserin and seganserin. With risperidone in the MES test, doses 2.3 to 3.0 times higher than in the 5-HTP test were required. In both tests, the smallest increases in ED_{50}'s over time were observed with ritanserin and the largest with risperidone so that ritanserin was the longest and risperidone the shortest acting compound.

Table 2 shows the results obtained in the 5-HTP test with a series of compounds and combination of compounds. Pirenperone, ketanserin, butanserin, and haloperidol inhibited and blocked 5-HTP-induced TWI. The ratios of the blocking to the inhibitory doses were 2.00 (haloperidol), 3.49 (butanserin), 4.08 (pirenperone), and 10.38 (ketanserin). Pimozide, prazosin, and tioperidone up to 10.0 mg/kg and the combination treatment of haloperidol 0.31 with prazosin 10.0 mg/kg were inactive. When ritanserin was combined with haloperidol 0.08 or with prazosin 0.04 mg/kg, in contrast to ritanserin alone, 5-HTP-induced TWI's were blocked at doses 3.9 to 5.2 times the inhibitory doses.

Table 3 shows the relative activity of the studied test compounds in a basic test for interactions with three neurotransmitters: the apomorphine test for dopamine D_2 antagonism, the tryptamine test for serotonin S_2 antagonism, and the norepinephrine test for α_1 adrenergic blocking activity. These relative activities are compared with the ability of the test compounds to block 5-HTP-induced TWI. The lowest ED_{50} obtained in the ATN test is symbolized by + + + +. All other ED_{50}'s are compared with this lowest ED_{50} and represented by + + + + if equipotent (ratio $ED_{50} < 2.0$), or less potent, i.e., + + + (ratio 2.1–8.0), + + (ratio 8.1–32.0), + (ratio 32.1–128), or inactive, 0 (ratio > 128). Very potent blockade of 5-HTP-induced TWI occurred in all instances of very potent tryptamine antagonism when this was associated to an additional potent activity on either dopamine or norepinephrine receptors.

DISCUSSION

Previous studies have documented the relation between 5-HTP- or MES-induced TWI in rats and the activation of serotonin receptors [Colpaert and Janssen, 1983; Goodwin and Green, 1985; Green et al., 1983; Niemegeers et al., 1983].

The present study confirms the high sensitivity of either challenge to serotonin S_2 antagonists, but it also provides the opportunity for a more direct comparison of the equivalence of both stimuli. With the selective serotonin S_2 antagonists ritanserin and seganserin [Colpaert et al., 1985; Janssen, 1985; Leysen et al., 1985], the doses inhibiting

TABLE 3. Symbolization of the Results of the ATN Test in Rats in Comparison With the Ability to Block 5-HTP-Induced TWI*

Compounds	Apomorphine Dopamine D_2 antagonism	Tryptamine Serotonin S_2 antagonism	Norepinephrine α_1 Adrenergic blocking activity	5-HTP Blockade
Prazosin	0	0	+ + + +	0
Tioperidone	0	0	+ + + +	0
Ritanserin	0	+ + + +	0	0
Seganserin	0	+ + + +	0	0
Pimozide	+ + + +	0	0	0
Haloperidol	+ + + +	+	0	+
Butanserin	0	+	+ + + +	+
Ketanserin	0	+ + + +	+ + +	+ + + +
Pirenperone	+ + + +	+ + + +	+ +	+ + + +
Risperidone	+ + +	+ + + +	+ + +	+ + + +
Haloperidol + prazosin	+ + + +	0	+ + + +	0
Haloperidol + ritanserin	+ + + +	+ + + +	0	+ + + +
Prazosin + ritanserin	0	+ + + +	+ + + +	+ + + +

*Symbols: The lowest ED_{50} of each compound is symbolized by + + + +. Other ED_{50}'s are compared with the lowest ED_{50} and represented by + + + + if equipotent (ratio ED_{50}'s < 2.0) or less potent + + + (ratio 2.1–8.0), + + (ratio 8.1–32), + (ratio 32.1–128), or inactive 0 (ratio > 128).

5-HTP- and MES-induced TWI were low and practically identical. Complete blockade of TWI in the MES test required slightly higher dose levels, but complete blockade of TWI in the 5-HTP test did not occur up to the very high dose of 10.0 mg/kg. In contrast, the potent serotonin S_2 antagonist risperidone [Janssen et al., 1988] at all testing intervals completely blocked the MES and the 5-HTP responses. Risperidone, in addition to blockade of S_2 receptors, is also a potent dopamine and norepinephrine receptor blocker. Awazi and Goldberg [1978], Everett [1979], and Van Praag [1983] have shown systemic injections of 5-HTP to increase not only the synthesis of serotonin, but also the turnover of dopamine and norepinephrine. Furthermore, it has been suggested that a portion of the exogenously administered 5-HTP may enter catecholaminergic terminals in which it undergoes a decarboxylation to 5-HT with resultant displacement of the endogenous catecholamine [Matthews and Smith, 1980; Handley and Brown, 1982]. It was thus possible that the additional activities of risperidone (dopamine and norepinephrine receptor blockade) were responsible for the observed difference between risperidone and the two selective S_2 antagonists ritanserin and seganserin. Therefore, antagonists of different types were selected on the basis of results obtained in the ATN test and included compounds acting exclusively as norepinephrine antagonists (prazosin and tioperidone) and as dopamine antagonists (pimozide and, up to a certain dose level, haloperidol).

Treatment with these compounds and various combinations produced results that are consistent with a clear requirement for blockade of 5-HTP-induced TWI. Activity on S_2 receptors is essential but should be associated with activity on dopamine D_2 or α_1 receptors. This is obtained with a single compound when it is a nonselective serotonin S_2 antagonist, used at a dose with associated dopamine and/or norepinephrine antagonism (risperidone, ketanserin, pirenperone). This is also obtained when ritanserin is combined with a low-dose haloperidol or prazosin.

Based on these results, it appears that there is an additional dopaminergic and/or noradrenergic component in 5-HTP-induced TWI, but not in MES-induced TWI. Both forms of TWI are nevertheless due primarily to a central serotonin S_2-receptor activation. Arguments in favour of this statement include the observations that 1) high correlations exist between the

antagonism of the 5-HTP- and MES-induced TWI and ^{3}H-spiperone binding in the frontal cortex of rats [Leysen and Tollenaere, 1982; Niemegeers et al., 1983]; 2) peripheral serotonin S_2-antagonists and putative S_1-agonists cannot antagonize both forms of TWI [Matthews and Smith, 1980; personal observations]; and 3) a perfect rank correlation (rs = 1.00) was found between the ability of the six serotonin S_2-antagonists to antagonize 5-HTP-induced TWI and tryptamine-induced bilateral clonic seizures of the forepaws and coarse body tremors, two central S_2-mediated effects [Niemegeers et al., 1977; Leysen and Tollenaere, 1982]. Furthermore, high correlations between the antagonism of 5-HTP- and MES-induced TWI (rs = 0.94, n = 21) [Niemegeers et al., 1983] and the antagonism of mescaline and tryptamine (rs = 0.92, n = 52) [Niemegeers et al., 1983] have been reported. It is not surprising that the catecholamines released by 5-HTP may have a role in the 5-HTP-induced TWI, as a variety of treatments can elicit the TWI behavior [Handley and Singh, 1986].

In the present study, a relatively high dose of 5-HTP was used. It could be argued that the association of lower doses of 5-HTP with the decarboxylase inhibitor carbidopa could be more selective for central serotonergic activation. On the basis of available literal data [Goodwin et al., 1983; Goodwin and Green, 1985], the effects of compounds are described exclusively as significant reductions rather than in terms of blockade. Theoretically, when a peripheral decarboxylation of 5-HTP is presented and more 5-HTP is available for central effects, it is expected that the catecholaminergic component will appear, as observed when 320 mg/kg of 5-HTP alone is used. Furthermore, it has been demonstrated that a too-high dose of carbidopa by itself can reduce twitching [Handley and Singh, 1986].

In conclusion, the present study indicates that the head twitch response to 320 mg/kg i.p. of 5-HTP is predominantly, but not entirely, serotonergic and therefore offers the possibility of differentiating between selective (inhibition of 5-HTP-induced TWI) and nonselective (blockade of 5-HTP-induced TWI) serotonin S_2 antagonists.

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