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RU 24969-produced adipsia and hyperlocomotion: Differential role of $5HT_{1A}$ and $5HT_{1B}$ receptor mechanisms

Dane Aronsen, Jeremy Webster, Susan Schenk *

School of Psychology, Victoria University of Wellington, P.O. Box 600, Wellington, New Zealand

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ABSTRACT

RU 24969 is a widely used, but non-selective, 5-$HT_{1B/1A}$ agonist that decreases fluid consumption and increases forward locomotion. The mechanism underlying these behavioural responses is not, however, well understood. Accordingly, effects of the selective 5-HT_{1A} and 5-HT_{1B} antagonists, WAY 100635, and GR 127935, respectively, on these two responses to RU 24969 were determined. RU 24969 (0.03–3.0 mg/kg, s.c.) dose-dependently decreased water consumption in water deprived rats. This effect was attenuated by GR 127935 (3.0 mg/kg), but not by WAY 100635 (1.0 mg/kg). RU 24969 (0.3–3.0 mg/kg) dose-dependently increased forward locomotion but a higher dose was required to produce this response than the adipsic response. The increased locomotor response was attenuated by WAY 100635 (1.0 mg/kg), but not GR 127935 (3.0 mg/kg). These results suggest that RU 24969-induced adipsia is mediated by 5-HT_{1B} mechanisms, while RU 24969-induced hyperlocomotion is mediated by 5-HT_{1A} mechanisms.

1. Introduction

Progress in understanding the role of the serotonin (5-HT) receptors in behaviour has been limited by the lack of selective pharmacological ligands. Thus, while 15 receptor subtypes from 7 receptor families, and their neural localisation, have been identified (Hoyer et al., 1994), ascribing functions to these receptor subtypes has proven difficult.

The 5-HT_{1B} receptor is of particular interest because of its widespread distribution in the brain (Sari, 2004) and its purported modulation of a large number of neurochemical systems. A substantial amount of research has focused on the impact of 5-HT_{1B} activation on GABA and glutamate release. Specifically, 5-HT_{1B} receptors are located presynaptically on GABA and glutamate terminals, and their activation inhibits release of these neurotransmitters (Sari, 2004). This function provides a mechanism for 5-HTergic modulation of release of these abundant neurotransmitters, and widespread effects on downstream neurotransmitter systems and behaviour.

Unfortunately, agonists for the 5-HT_{1B} receptor are generally non-selective. A case in point is one of the most commonly tested ligands, RU 24969 (5-Methoxy-3-(1,2,5,6-tetrahydro-4-pyridinyl)-1H-indole). This drug is a preferential 5-HT_{1B} agonist (Ki = 0.38 nmol), but also displays appreciable affinity for the 5-HT_{1A} receptor (Ki = 2.5 nmol) and has low affinity for other receptor sites in the brain (Peroutka, 1986; Wolf and Kuhn, 1991). Both the 5-HT_{1B} and 5-HT_{1A} receptors are negatively coupled to adenylate cyclase through a G-protein (Barnes and Sharp, 1999; Wang et al., 2013).

Hyperlocomotion is a prominent effect of RU 24969. This behavioural response was not attenuated by depletion of brain 5-HT, suggesting a post synaptic mechanism (Cheetham and Heal, 1993). Studies in mice have generally attributed RU 24969-induced hyperlocomotion to 5-HT_{1B} mechanisms because it was selectively attenuated by pretreatment with 5-HT_{1B}, but not 5-HT_{1A}, antagonists (Cheetham and Heal, 1993; Shanahan et al., 2009). In the rat, however, there is a lack of consensus as to whether this effect is due to 5-HT_{1A} or 5-HT_{1B} activation because, in different circumstances, RU 24969-induced hyperlocomotion has been blocked by both 5-HT_{1A} and 5-HT_{1B} antagonists (Chaouloff et al., 1999; Kalkman, 1995; O'Neill and Parameswaran, 1997).

Another response to RU 24969 is a decrease in fluid consumption. RU 24969 non-selectively reduced intake of both water and sweetened ethanol (Silvestre et al., 1998), responding maintained by water in water-deprived rats (Carli et al., 1988), and the time spent drinking sweetened condensed milk (Simansky and Vaidya, 1990). To our knowledge there have not been any pharmacological studies to determine whether this decrease in fluid consumption is due to effects at 5-HT_{1A} or 5-HT_{1B} receptors.

One way to tease apart the various responses and to attribute them to one or another receptor subtype is to make use of the more selective antagonists. Fortunately, selective pharmacological antagonists for the 5-HT_{1A} and 5-HT_{1B} receptors are available. WAY 100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride) is a 5-HT_{1A} antagonist with over 100-fold selectivity relative to other neurotransmitter receptors (Forster et al., 1995). GR 127935 (N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-1-1′-biphenyl-4-carboxamide) is a 5-$HT_{1B/1D}$ antagonist

* Corresponding author. Tel.: +64 4 4636034; fax: +64 4 4635402.
E-mail address: Susan.Schenk@vuw.ac.nz (S. Schenk).

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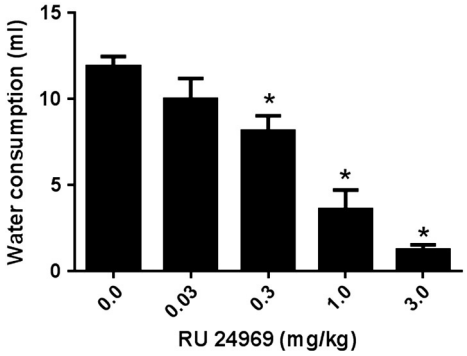

Fig. 1. Effect of RU 24969 on water consumption, $^{*}p < 0.05$.

with selectivity for the 5-HT_{1B} receptor (pKi = 9.0) and 5-HT_{1D} receptor (pKi = 8.6) over the 5-HT_{1A} receptor (pKi = 7.2) and other 5-HT receptors (Price et al., 1997). In the present study we show that two behavioural responses to RU 24969 can be dissociated pharmacologically.

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats were bred in the Victoria University of Wellington vivarium. They were housed in groups of 4 in a temperature- (19–21 ºC) and humidity- (55%) controlled environment until they reached weights of 300–350 g, after which they were housed individually for two days prior to testing. The housing colony was maintained on a 12 h light/dark cycle (lights on at 7.00 am) and all tests were conducted during the light portion of the cycle. Food and water were freely available except during testing.

2.2. Apparatus and procedure

Water consumption was measured in the home cage. Water bottles were removed and 23.75 h later the 5-$HT_{1B/1A}$ agonist, RU 24969 (0.0–3.0 mg/kg, s.c.; N = 10 per group), was administered. The water bottles were reintroduced 15 min after the RU 24969 injection. Consumption was measured for a 30 minute period. Fluid consumption was determined by weighing water bottles before and after the test. Separate groups (N = 6–9 per group) were tested in the same manner to assess the contribution of 5-HT_{1A} or 5-HT_{1B} mechanisms. Either the 5-HT_{1A} antagonist, WAY 100635 (0.0, 1.0 mg/kg, s.c.), or the 5-$HT_{1B/1D}$ antagonist, GR 127935 (0.0, 3.0 mg/kg, s.c.) was administered 15 min before RU 24969 (1.0 mg/kg, s.c.). These doses were chosen for their documented efficacy in blocking 5-HT_{1A} and 5-HT_{1B} effects, respectively (Acosta et al., 2005; Fletcher and Korth, 1999).

Locomotor activity testing was conducted in clear Plexiglas chambers (Med Associates Inc., USA; model ENV-515) measuring 42 × 42 × 30 cm, set in sound-attenuating boxes. Forward locomotion was measured with two sets of 16 infrared beams and sensors spaced evenly along the sides of the chambers producing squares measuring 25 mm × 25 mm. The interruption of three adjacent beams (the approximate size of the body of a rat) was recorded as one activity count. A white noise generator was used during experiments to mask any outside noise, and chambers were washed with Virkon 'S' disinfectant (Southern Veterinary Supplies, NZ) after testing to control for olfactory confounds. Experiments were run in a dark room, except for a red light that was used to illuminate the room during drug administrations.

Rats were placed in the testing chamber for 30 min, followed by an injection of RU 24969 (0.0–3.0 mg/kg, s.c.; N = 8 per group), and activity was measured for 45 min post-injection. Separate groups (N = 6–12 per group) were placed in the activity monitoring chambers and 15 min later received either WAY 100635 (0.0, 1.0 mg/kg, s.c.) or GR 127935 (0.0, 3.0 mg/kg, s.c.), followed 15 min later by RU 24969 (3.0 mg/kg, s.c.). In order for the data to be directly comparable to the fluid consumption protocol, only data collected from 15 to 45 min following the injection of RU 24969 were analysed.

2.3. Drugs

RU 24969 (Tocris, New Zealand) and WAY 100635 (Tocris, New Zealand) were dissolved in sterilised saline. GR 127935 (Tocris, New Zealand) was dissolved in distilled water. All injections were a volume of 1.0 ml/kg. All drug doses were based on salt weights.

3. Results

Fig. 1 shows the effect of RU 24969 on water consumption. ANOVA confirmed an effect of dose ($F(4, 45) = 24.56$, $p < 0.001$), and post hoc Tukey analysis indicated that 0.3, 1.0, and 3.0 mg/kg significantly decreased water consumption. Effects of the antagonists on RU 24969-produced adipsia are presented in Fig. 2.

Analysis of the effect of WAY 100635 (dose RU 24969 × dose WAY 100635) revealed a main effect of RU 24969 ($F(1,26) = 26.95$, $p < .001$), but no effect of WAY 100635 ($F(1,26) = 0.016$, *ns*) or an interaction ($F(1,26) = 0.83$, *ns*). In contrast, analysis of the effect of GR 127935 (dose RU 24969 × dose GR 127935) revealed an effect of GR 127935 ($F(1,24) = 4.55$, $p = 0.043$), an effect of RU 24969 ($F(1,24) = 29.44$, $p < 0.001$) and an interaction ($F(1,24) = 9.02$, $p = 0.006$). Tukey post hoc comparisons confirmed that GR 127935 significantly reduced RU 24969-produced adipsia ($p < 0.05$).

Fig. 3 shows that RU 24969 increased locomotor activity ($F(3,28) = 8.15$, $p < 0.001$). Post hoc Tukey analysis showed the dose of 3.0 mg/kg was the only dose that significantly increased total forward locomotion. Effects of the antagonists on RU 24969-produced hyperlocomotion are presented in Fig. 4.

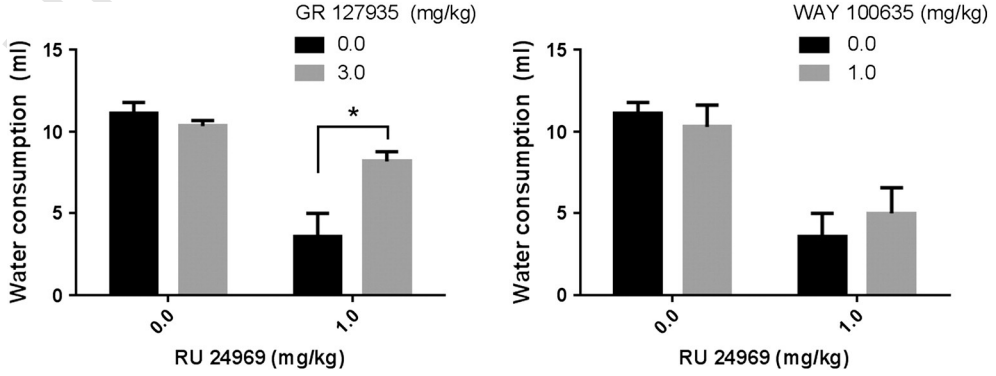

Fig. 2. Effect of GR 127935 (left) or WAY 100635 (right) on RU 24969-produced adipsia.

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