Pharmacology Biochemistry & Behavior, Vol. 17, pp. 1155–1159, 1982. Printed in the U.S.A.

Neuropharmacological Profile of R 18 503-Induced Wet-Shake Behavior in the Rat: Noradrenergic and Opiate Components

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Received 16 November 1981

ASHTON, D. AND A. WAUQUIER. *Neuropharmacological profile of R 18 503-induced wet-shake behaviour in the rat: Noradrenergic and opiate components.* PHARMAC. BIOCHEM. BEHAV. 17(6) 1155–1159, 1982.—R 18 503 (α-(*p*-chlorophenyl)-β,β-dimethylimidazole-1-ethanol) 40 mg/kg IP produced a mean of 168 (SEM±4) wet shakes in the 35 min following its administration to 100 g male Wistar rats. No other behavioural abnormalities were seen at this dose. The wet-shakes were not caused by reduced body temperature. However, 20–30 min after R 18 503, body temperature was significantly elevated in comparison with saline treated controls. This supports a role for wet-shakes in thermogenesis in the rat, but suggests that R 18 503-induced wet-shakes are not adapted towards the maintenance temperature homeostasis. R 18 503-induced wet-shakes were potently antagonized by 5 narcotic analgesics, but not by aspirin-like drugs. A further 18 non-narcotic compounds including putative serotonin antagonists, neuroleptics and compounds thought to act on the α-adrenergic system, also antagonized R 18 503-induced wet-shakes. Spectral map analysis showed a close link between ED_{50}-values of the non-narcotic R 18 503 wet-shake antagonists and their ED_{50}'s to antagonize noradrenaline-induced lethality; this was further confirmed by a highly significant Spearman Rank correlation between the two tests ($r_s=0.77, p<0.001$). However, the spectral mapping and Spearman correlation analysis showed no relationship between antagonism of R 18 503-induced wet-shakes and antagonism of apomorphine-induced stereotypy, tryptamine-induced bilateral forepaw clonus, mescaline-induced head twitches, and 5 HT-induced contractions of rat caudal artery. It is postulated that R 18 503-induced wet-shakes result from an interaction between the opiate system and the α-adrenergic system.

Narcotic analgesics Noradrenaline Serotonin Narcotic withdrawal Rat behavioural tests Neuroleptics

WET-DOG SHAKE behaviour in rats has been reported after many different pharmacological manipulations. It is a component of narcotic analgesic withdrawal [20] and precipitated narcotic abstinence [5]. Wet-shaking is produced by the injection of some hallucinogens [8], and has been considered as a quantitative behavioural model for central serotonergic transmission [3]. Other compounds producing wet-shakes include endorphins [6], kainic acid [4] and carbachol chloride [24]. Thyrotrophin releasing hormone-induced wet-shakes have been the subject of an extensive pharmacological study by Kruse and Moeller [16]. In addition wet-shakes are a component of kindling development during amygdaloid stimulation [1] and during cobalt-induced experimental epilepsy in rats [7]. Three other experimental compounds, AG 3–5, a tetrahydro-pyrimidine derivative [26]; Sgd-8473, a benzylidene-amino-oxycarburic acid derivative [12], and isosorbide dinitrate (ISDN), a coronary dilator [15] have also been reported as wet-shake inducers.

We recently observed that R 18 503 (α-(*p*-chlorophenyl)-β, β-dimethylimidazole-1-ethanol) induced frequent (approx. 150 per 30 min) and severe wet-shakes in the absence of any other behavioral manifestations after intraperitoneal or intravenous administration in rats. The present study focuses on the neuropharmacological antagonism of these wet-shakes in order to facilitate comparisons between R 18 503 and other compounds which mimic some aspects of the precipitated abstinence syndrome or induce the quasi-morphine withdrawal syndrome [9].

METHOD

A wet-shake was defined as an abrupt, rapid shaking of the head, forelimbs, thorax and upper abdomen. All experiments were done in overnight food-deprived 100 (±10) g male Wistar rats of the laboratory strain. All experiments were carried out between 20–25° C. During each session of 35 min the cumulative number of wet-shakes was tabulated each 5 min for 5 animals simultaneously by an observer who was blind to treatment.

R 18 503 dissolved in distilled water at a concentration of 4 mg/ml was injected intraperitoneally so as to give a dose of 40 mg/kg. Challenge drugs were given at times given in Table 1. Parachlorophenylalanine was given IP 250 mg/kg 48 hours and 24 hours before R 18 503.

The mean total number of wet-shakes over 35 min in 88 control animals after injection of 40 mg/kg R 18 503 IP was 168 (±4). Only one animal out of 88 had a wet-shake count below 113; values lower than 113 were therefore considered as significant inhibition. ED_{50}-values and 95% confidence limits were then calculated according to Finney [11].

In addition a dose-response curve for R 18 503 was made and oesophageal temperature was measured in rats treated with 40 mg/kg R 18 503.

RESULTS

Dose-Response Curve R 18 503

Figure 1 shows cumulative number of wet-shakes elicited after 10, 20, 40, and 160 mg/kg of R 18 503. Wet-shaking began between 5 and 10 minutes after IP administration of R 18 503. There was a dose-related increase in wet-shakes) except for the highest dose tested. The wet-shaking was maximal after 40 mg/kg. However, the 160 mg/kg dose of R 18 503 was a suspension and may thus have been more slowly absorbed. Wet-shaking continued for 2 to 3 hours.

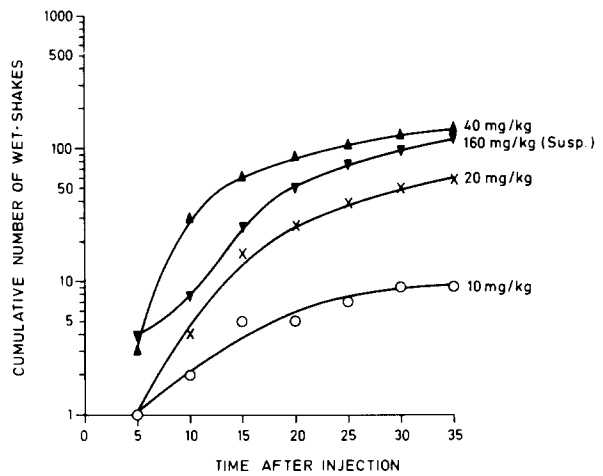

FIG. 1. Cumulative median number of wet-shakes scored over 35 min in rats injected with different doses of R 18 503. ○–○ 10 mg/kg, X–X 20 mg/kg, ▼–▼ 160 mg/kg, ▲–▲ 40 mg/kg (160 mg/kg given as suspension). n=10 animals per dose.

Antagonism of R 18 503 Wet-Shakes

Table 1 lists ED_{50}-values (±95% CL) of a large series of compounds to antagonize R 18 503 wet-shaking. Of 28 compounds, 22 were active and 6 inactive. The narcotic analgesics sufentanil, fentanyl, alfentanil, morphine and piritramide were potent antagonists and their ED_{50} for blockade of tail-withdrawal were higher than the ED_{50}'s for R 18 503 antagonism [13].

In contrast, the non-narcotic analgesics aspirin, indomethacin and suprofen were inactive at the tested doses.

The relatively specific dopamine blocking neuroleptic compounds (haloperidol, pimozide and spiperone) antagonized the R 18 503-induced wet-shakes at relatively high doses, however, the neuroleptics with pronounced noradrenergic blocking action (azaperone, pipamperone, milenperone and declenperone) were also effective R 18 503 antagonists. The putative serotonin antagonists mianserin, metergoline, cinanserin, methysergide, cyproheptadine and pizotifen were also active at high doses. However, PCPA 250 mg/kg IP 48 and 24 hours before R 18 503 was without effect. Clonidine, phenoxybenzamine and prazosin were also active. Tetrabenazine antagonized the wet-shakes (ED_{50} 1.16 mg/kg) at low doses.

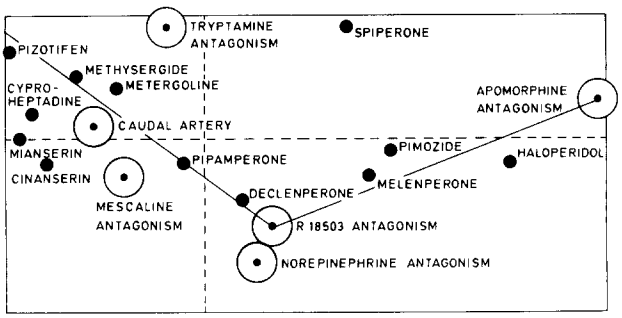

FIG. 2. Principal component mapping illustrating the spatial configuration of 6 pharmacological tests (antagonism of apomorphine stereotypy, antagonism of tryptamine-induced bilateral forepaw clonus, antagonism of 5-HT-induced contractions of rat caudal artery, antagonism of mescaline-induced head-twitches, antagonism of norepinephrine-induced lethality, and antagonism of R 18 503-induced wet-shakes) and 12 compounds in a plane defined by two components explaining 72% (abscissa) and 14% (ordinate) of the variance.

Spectral Mapping

In order to compare the neuropharmacological spectrum of R 18 503 antagonism with other pharmacological tests spectral mapping [18] was carried out for the 12 compounds for which complete ED_{50} profiles were available. These tests were apomorphine, tryptamine and norepinephrine antagonism [21], antagonism of mescaline-induced head-twitches (Niemegeers, personal communication), and antagonism of 5 HT-induced contractions of isolated rat caudal arteries [25]. The resulting spectral map is shown in Fig. 2. The tryptamine caudal artery, and mescaline tests appear as one cluster, suggesting that this is the serotonergic component of the spectral map. Apomorphine antagonism occurs at the other pole of the figure. R 18 503 and norepinephrine antagonism appear to be very closely linked. This result was further confirmed by a series of Spearman rank-order correlation analyses. R 18 503 vs rat caudal artery $r_s=0.176$ $p>0.05$, R 18 503 vs mescaline antagonism $R_s=0.350$ $p>0.05$, R 18 503 vs tryptamine antagonism $r_s=0.25$ $p>0.05$ and R 18 503 vs norepinephrine antagonism $r_s=0.77$ $p<0.001$ again showing the relationship between the non-narcotic R 18 503 antagonists and noradrenaline.

R 18 503 and Temperature

Oesophageal temperature was measured in ten 100 g male rats divided into 2 groups of 5 rats. Ambient temperature was 20.7° C. After a 20-minute acclimatization period rats were injected with R 18 503 40 mg/kg IP or saline. Oesophageal temperature was measured every 5 min for 35 min. The results are shown in Fig. 3. There was a drop in oesophageal temperature in both NaCl and R 18 503 groups immediately

TABLE 1

ED_{50}-VALUES (±95% CONFIDENCE LIMITS) OF COMPOUNDS TO ANTAGONIZE R 18 503 40 mg/kg IP INDUCED WET-SHAKES

Compound	Route	Time (min)	ED_{50} (mg/kg)	Lower limit	Upper limit
Sufentanil	SC	−30	0.0015	0.00086	0.0025
Fentanyl	SC	−60	0.011	0.007	0.013
Clonidine	SC	−60	0.049	0.037	0.064
Alfentanil	SC	−30	0.051	0.029	0.088
Spiperone	SC	−30	0.32	0.18	0.55
Morphine	SC	−30	0.38	0.30	0.51
Prazosin	SC	−60	0.50	0.08	3.14
Milenperone	SC	−30	0.52	0.30	0.90
Haloperidol	SC	−30	0.64	0.37	1.10
Piritramide	SC	−30	1.03	0.60	1.78
Tetrabenazine	SC	−120	1.16	0.67	2.04
Declenperone	SC	−30	1.51	0.88	2.61
Metergoline	SC	−30	2.08	1.21	3.59
Azaperone	SC	−30	2.10	1.61	2.73
Pipamperone	SC	−30	2.68	2.06	3.51
Mianserin	SC	−30	3.19	1.85	5.51
Pimozide	SC	−30	3.92	2.27	6.75
Indomethacin	OR	−60	*(10)		
Suprofen	OR	−60	*(10)		
Domperidone	SC	−30	*(10)		
Xylamidine	SC	−30	*(10)		
Cyproheptadine	SC	−30	11.3	6.54	19.5
Methysergide	SC	−30	16.7	9.7	28.9
Cinanserin	SC	−30	19.8	11.5	25.9
Pizotifen	SC	−30	20.8	12.0	35.9
Phenoxybenzamine	SC	−30	37.4	28.6	48.9
Aspirin	OR	−60	*(40)		
Parachlorophenyl-alanine	IP		*(250×2)		

*Not active at dose in parentheses.

after injection, thereafter, the control group temperature returned to baseline. However, after the onset of wet-shaking the (±5 min) temperature in the R 18 503 group began to climb; becoming significantly different from controls at 20, 25 and 30 min after injection (Mann-Whitney U-test, Two-tailed).

DISCUSSION

R 18 503 produces wet-shakes in rats in the absence of any other obvious behavioural change at 40 mg/kg IP. Several authors have suggested that wet-shaking is a form of adaptive behaviour which enables the rat to maintain body temperature [28]. Thus experimental or pharmacological interventions leading to a rise or fall in body temperature will respectively lead to decrease or increase in wet-shake behaviour [27]. For instance, kainic acid induced wet-shakes begin when the body temperature fall reaches a peak (up to 3° C); and finish as the body temperature begins to rise [4]. It is clear that the fall in temperature after R 18 503 injection and before the onset of wet-shakes is small (±0.4°C) and that the temperature fall also occurs in saline treated controls. Figure 2 also shows that during the time of peak wet-shaking the body temperature of R 18 503 treated rats is significantly higher than that of saline treated controls. This lends support to the hypothesis that wet-shake behaviour is a thermogenic mechanism in the rat; and suggests that R 18 503-induced wet-shaking is forced and is not-adapted towards the preservation of normal temperature. This does not preclude that R 18 503 influences the animals' temperature sensing system, or the neurones controlling temperature behaviour, in such a way that they perceive a temperature drop, and instigate the wet-shake behaviour.

The neuropharmacological profile of R 18 503-induced wet-shake antagonism generated several hypotheses as to its neuronal effects. Notable, was the potency of the narcotic analgesics, whose ED_{50}-value for antagonism of R 18 503 was below their ED_{50} for producing analgesic effects in the tail-withdrawal test.

Wet shakes are a component of narcotic withdrawal [20] and precipitated abstinence [5], though their frequency is low (max 15 per 30 min) suggesting that the wet-shakes are unimportant for thermogenesis. The failure of the aspirin-like drugs to antagonize R 18 503-induced wet-shakes suggests that prostaglandin biosynthesis is not involved in R 18 503 effects. In this respect it is interesting to note that wet-shakes are produced by intracerebro ventricular administration of methionine enkephalin, and that these are inde-

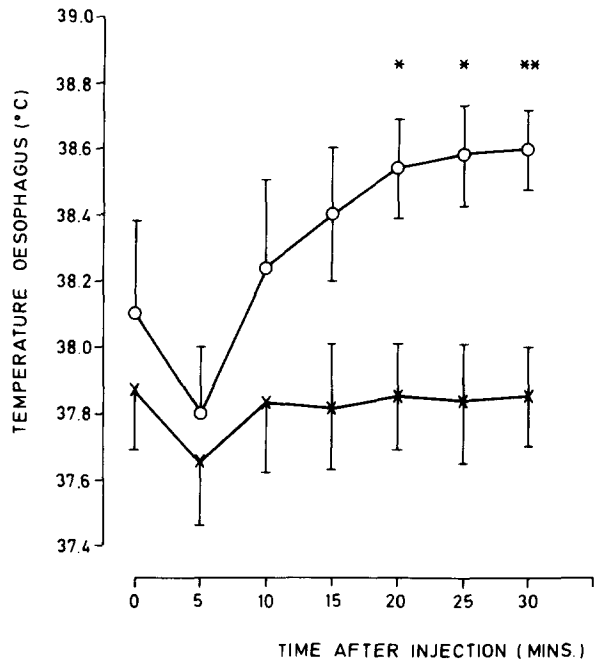

FIG. 3. Mean oesophageal temperature (±SEM) measured after injection of R 18 503 40 mg/kg IP (○–○) or saline (X–X). Statistical. significance measured with Mann-Whitney U-test, two-tailed probability *=$p<0.05$; **=$p<0.01$. Ambient temperature 20.7° C.

pendent of serotonin [10]. This suggests that one component of R 18 503-induced wet-shake behaviour may reside in interference with an endogenous opiate system, or that a narcotic analgesic induces functional effects on other transmitter systems. Narcotic analgesics are also antagonists of kainic acid induced shaking [14] and thyrotropin releasing hormone-induced shaking [16] at non-catatonic doses. The effects of naloxone given alone and in combination with fentanyl on R 18 503-induced wet-shakes are described in a previous article [2]. However naloxone on its own was without effect at doses between 0.1 and 10 mg/kg SC.

Various non-antinociceptive compounds were also effective antagonists of R 18 503-induced wet-shakes. Amongst these was a group of putative serotonin antagonists, but the overall profile of R 18 503 antagonists and their ED_{50}-values showed no correlation with antagonism of mescaline-induced head-twitches, or with antagonism of 5 HT-induced contractions of the caudal artery, or with antagonism of tryptamine-induced bilateral forepaw clonus. All three of these measures correlate with the IC_{50}-values of drugs to displace ^{3}H-spiperone from 5-HT_2 receptors in rat frontal cortex [19]. Principal component analysis of the profiles of compounds to antagonize apomorphine stereotypy, noradrenaline mortality, tryptamine forepaw clonus, 5 HT-induced caudal artery contraction, and mescaline-induced head-twitches revealed a close association between antagonism of noradrenaline and antagonism of R 18 503. This was further strengthened by a highly significant Spearman-rank correlation between R 18 503 antagonism and noradrenaline antagonism.

The hypothesis that activity of the putative 5-HT antagonists is not related to serotonin, and that 5-HT is a trivial component of R 18 503-induced wet-shakes is supported by the high ED_{50}-values of the putative serotonin antagonists against R 18 503 in comparison to other pharmacological tests of serotonergic function. R 18 503 does not bind to the $5HT_2$ receptor labelled by ^{3}H-spiperone in the rat frontal cortex (J. Leysen, personal communication) and the failure of PCPA to alter the number of R 18 503-induced wet-shakes suggests that release of endogenous 5HT is an unimportant mechanism of R 18 503 wet-shake induction. Receptor binding reveals that most putative serotonin antagonists effective against R 18 503 have significant and specific effects on α_1- or α_2-noradrenergic receptors [19]. The importance of the noradrenergic components of R 18 503 antagonism is also demonstrated by the high potency of clonidine, prazosin and tetrabenazine. The high ED_{50} of phenoxybenzamine is probably explained by its low brain penetration, in addition its activity at α_1 and α_2 receptors may result in antagonism of its own effects at high doses (Van Neuten, personal communication).

In summary, R 18 503 produces wet-dog shakes in the rat in the absence of other obvious behaviour abnormalities. These wet-shakes are antagonized by manipulations which block opiate receptors or α-noradrenergic receptors, and by depletion of catecholamines. The wet-shakes do not appear to be a behavioural response to a reduced temperature, but do have thermogenic effects. Serotonin appears to have a relatively unimportant role in R 18 503 wet-shakes. The potent activity of narcotic analgesics suggests some relationship between R 18 503-induced wet-shakes and the wet-shakes seen in narcotic withdrawal. It is interesting to not that prior administration of alpha methyl paratyrosine specifically antagonizes the wet-shakes produced during precipitated narcotic abstinence, and that PCPA was without effect [24]. In addition, NA levels are significantly higher in the hypothalamus during narcotic withdrawal [22]. Further studies are necessary to demonstrate the site and biochemical nature of R 18 503's effects; and to further explore the relationship between opiate and α-adrenergic effects.

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