Modafinil increases histamine release in the anterior hypothalamus of rats

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Abstract

Modafinil, (RS)-2-(Diphenylmethylsulfinyl)acetamide, is a well known wake promoting drug used for the treatment of narcolepsy. We investigated the effect of modafinil on the hypothalamic histamine release in the anesthetized rat using in vivo microdialysis. Modafinil (150 mg/kg, i.p.) increased histamine release by 150% of the basal release. The intracerebroventricular (i.c.v.) injection of modafinil (1 nmol) also increased histamine release, however, when modafinil (1 nmol) was injected directly into the tuberomammillary nucleus, a limited region where cell bodies of the histaminergic neurons are located, histamine release was not altered. These observations suggest that modafinil may promote waking via the activation of the histaminergic system, although it does not appear to be a direct pharmacological target of modafinil.

Keywords: Modafinil; Histamine; Microdialysis; Anterior Hypothalamus; Tuberomammillary nucleus; Arousal

Modafinil, (RS)-2-(Diphenylmethylsulfinyl)acetamide, promotes wakefulness without tolerance and sensitization [1, 16] unlike amphetamine and is used for the treatment of narcolepsy. A previous study reported that the patterns of Fos expression by modafinil differed from one of amphetamine [3], which suggests modafinil and amphetamine act via the different mechanisms. However, the mode of action of modafinil remains unclear.

The newly discovered neuropeptides orexin-A and -B [13] were suggested to play important roles in the regulation of the arousal state. Central administrations of them produce wakefulness [5], while disruptions of the orexinergic system [2, 6] exhibit a phenotype similar to human narcolepsy. Based on the finding that modafinil activates orexin-containing neurons [2, 14], the orexinergic system may be a target for the action of modafinil.

Histamine is one of the important neurotransmitters that control the sleep–waking stage. Mochizuki et al. [10] showed that histamine released from the anterior hypothalamus in rats was higher during the dark phase than during the light phase, supporting the idea that histamine is a waking amine [21]. Several lines of evidence have shown the functional connection between the histaminergic system and the orexinergic system in the regulation of the arousal state. The orexinergic neurons densely innervate the tuberomammillary nucleus (TM), a limited region where cell bodies of the histaminergic neurons are located [12, 19], and orexin-1 and orexin-2 receptors are expressed on the TM neurons [4]. In addition, both orexin-A and -B depolarized the TM neurons [4, 19], and increased histamine release from the hypothalamus [7, 8] and the frontal cortex [7] when they were centrally injected. Huang et al. [7] also reported that orexin-A had no effect on the sleep–waking stage in histamine H1 receptor knockout mice. Taken together with these observations, orexin may express its wake-promoting effect through the activation of the histaminergic system. Moreover, Scammell et al. [14] showed that modafinil induces Fos expression in both the TM and orexin neurons of rats, suggesting that modafinil may act through the histaminergic system as well as the orexinergic system.

Therefore, in the present study, we examined the effect of modafinil on hypothalamic histamine release using in vivo microdialysis, and whether the histaminergic system is a direct target of modafinil.

Male Wistar strain rats (Japan SLC, Shizuoka, Japan) weighing 180–260 g were used. The rats were kept two to a cage on a 12:12 h light/dark schedule (lights on, 07:00 h to 19:00 h). They had free access to standard pelleted chow.
The effects of modafinil (150 mg/kg, i.p.) on the release of histamine from the rat anterior hypothalamus. The mean basal histamine releases of rats treated with i.p. modafinil and that of controls were 0.074 ± 0.012 pmol/20 μl and 0.069 ± 0.010 pmol/20 μl, respectively. The average mean value in the first three samples is taken as 100%. Values are presented as percentages of the mean basal release and SEM. **P < 0.01, *P < 0.05 compared with the basal release of each group.

Fig. 1. The effects of modafinil (150 mg/kg, i.p.) on the release of histamine from the rat anterior hypothalamus. The mean basal histamine releases of rats treated with i.p. modafinil and that of controls were 0.074 ± 0.012 pmol/20 μl and 0.069 ± 0.010 pmol/20 μl, respectively. The average mean value in the first three samples is taken as 100%. Values are presented as percentages of the mean basal release and SEM. **P < 0.01, *P < 0.05 compared with the basal release of each group.

Fig. 2. The effects of modafinil (1 nmol, i.c.v) on the release of histamine from the rat anterior hypothalamus. The mean basal histamine releases of rats treated with i.c.v. modafinil and that of controls were 0.062 ± 0.034 pmol/20 μl, respectively. The average mean value in the first three samples is taken as 100%. Values are presented as percentages of the mean basal release and SEM. **P < 0.01, *P < 0.05 compared with the basal release of each group.

On the experimental day, rats were anesthetized with urethane (1.2 g/kg, i.p.) and placed on a stereotaxic apparatus (Kopf Instrument, Tujunga, CA, USA). The microdialysis was carried out as described previously [8,11]. The microdialysis probe (MAB6, membrane length 2 mm, ALS/Microbiotech, Stockholm, Sweden) aimed at the anterior hypothalamus was inserted at coordinates: AP, −1.5; LM, −0.5; DV, 9.2 mm. Probes were perfused with artificial cerebrospinal fluid [11] at a rate of 1 μl/min for 2 h to stabilize the histamine release, and then dialysates were collected every 20 min. After collecting the first three samples for 1 h (baseline samples), rats were injected intraperitoneally with either modafinil (150 mg/kg) suspended in a solution of 0.3% carboxymethylcellulose in 0.9% saline, or vehicle at a volume of 6 ml/kg. Another group of rats were implanted with a guide cannula (22-gauge) into the third ventricle for i.c.v. injection, AP, −4.5; LM, 0; DV, 4.8 mm relative to the bregma and the skull surface, immediately before the dialysis probe was inserted [8]. For the intra-TM injection, a guide cannula was aimed at the TM, AP, −4.3; LM, −1.3; DV, 7.6 mm [11].

After baseline samples were collected, a 27-gauge injection cannula was inserted through the guide cannula for both central injections. Two minutes after the insertion, modafinil (1 nmol) included in a solution of 0.07 M 2,6-di-O-methyl-β-cyclodextrin (host:guest = 2:1) in 0.9% saline or vehicle was injected at a rate of 1 μl/min for 2 min. Dialysates were collected for 4 h after the drug administration. After all dialysates were recovered and the probe was removed, the rats were perfused with 0.9% saline through the left ventricle, followed by 10% formalin. Brains were removed and histological verification of probe placement was performed as described previously [8]. Histamine levels in dialysate fractions were determined by an HPLC-fluorometric method [20]. Since absolute basal release of histamine varied between subjects, the mean of the first three fractions were defined as the mean basal release and the following fractions were expressed as the percent of the mean basal release. The statistical differences between the relative value of the mean basal release and fractions for each group were analyzed using a one-way ANOVA with post-hoc LSD test.

Modafinil (n = 7) significantly increased histamine release about 2 h after drug administration by 150% of the basal release (F(14, 84) = 2.46, P < 0.01), whereas vehicle (n = 6) injection showed no effect (F(14, 42) = 0.54, P = 0.90) (Fig. 1). Similarly, histamine release increased about an hour after i.c.v. injection (n = 6, F(14, 70) = 3.14, P < 0.01). In control rats, histamine release was not changed (n = 5, F(14, 56) = 0.94, P = 0.52) (Fig. 2). On the other hand, no increase in the histamine release was produced by the intra-TM injection of modafinil (1 nmol) (n = 5, F(14, 56) = 1.78, P = 0.06), or by vehicle (n = 4,
Intra-TM injection did not increase histamine release (Fig. 3). Thus, we carried out the experiment by the injection of modafinil into the TM directly, to clarify modafinil. However, since modafinil can promote wakefulness in the orexin-2 receptor mutant dog [18] and modafinil has low affinity for orexin-1 receptors [17], the possibility that modafinil increases wakefulness in an orexin-independent manner cannot be ruled out. Modafinil reduces Fos expression in the ventrolateral preoptic area (VLPO) during the day [14]. The VLPO contains sleep-promoting neurons [9] and sleep-active neurons in the VLPO provide inhibitory GABAergic input to the TM [15]. Thus, modafinil may lower the activity of the VLPO neurons, which in turn disinhibit the histaminergic TM neurons and promote waking.

In conclusion, the histaminergic system, at least in part, plays a role in promoting wakefulness by modafinil. Further experiments to study the effect of modafinil on the histamine release in the orexin knockout mice are needed to clarify the importance of the orexinergic system in the activation of the histaminergic system by modafinil.

References


