

The Vigilance Promoting Drug Modafinil Increases Extracellular Glutamate Levels in the Medial Preoptic Area and the Posterior Hypothalamus of the Conscious Rat: Prevention by Local GABA_A Receptor Blockade

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The effects of modafinil on glutamatergic and GABAergic transmission in the rat medial preoptic area (MPA) and posterior hypothalamus (PH), are analysed. Modafinil (30–300 mg/kg) increased glutamate and decreased GABA levels in the MPA and PH. Local perfusion with the GABA_A agonist muscimol (10 μM), reduced, while the GABA_A antagonist bicuculline (1 μM and 10 μM) increased glutamate levels. The modafinil (100 mg/kg)-induced increase of glutamate levels was antagonized by local perfusion with bicuculline (1 μM). When glutamate levels were increased by the local perfusion with the glutamate uptake inhibitor L-trans-PDC (0.5 mM),

modafinil produced an additional enhancement of glutamate levels. Modafinil (1–33 μM) failed to affect [³H]glutamate uptake in hypothalamic synaptosomes and slices. These findings show that modafinil increases glutamate and decreases GABA levels in MPA and PH. The evidence that bicuculline counteracts the modafinil-induced increase of glutamate levels strengthens the evidence for an inhibitory GABA/glutamate interaction in the above regions controlling the sleep-wakefulness cycle.

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Modafinil, a (diphenyl-methyl)-sulfinyl-2-acetamide derivative (Modiodal®), has been successfully used as a vigilance enhancer devoid of the behavioural modifications, tolerance, and sensitisation observed with amphetamine (Touret et al. 1995). In addition, modafinil displays a different neurochemical profile of action from that of amphetamine and amphetamine-like psychostimulant drugs, suggesting that its awakening effect does not involve dopaminergic mechanisms (Ferraro et al. 1997a; 1997b; Duteil et al. 1990). However, although numerous morphological and biochemical studies have been performed to define the cellular mechanisms underlying the vigilance promoting action of the drug, its effect on the neurochemical parameters regulating wake-

fulness in sleep related brain areas remains still undefined.

The medial region of the hypothalamus contains a series of large nuclei including the medial preoptic area (MPA) and the posterior hypothalamus (PH). These relay nuclei have associational functions and project to the association cortex (including the anterior cingulate) and are involved in mediating generalised aspects of behavioural states and arousal as well as playing a key role in the initiation of motivated behaviours (Swanson 1987; Wayner et al. 1981). Furthermore, the PH is thought to contribute to the production of wakefulness and electroencephalographic desynchronization (Nitz and Siegel 1996) and the MPA is considered a crucial site for the regulation of sleep (Azuma et al. 1996).

A recent microdialysis study has shown that a vigilance enhancing dose of modafinil (100 mg/kg) reduces local extracellular GABA levels in the MPA and PH suggesting that GABA transmission in these subregions of the hypothalamus may regulate wakefulness and that the inhibition of extracellular GABA levels may be a target for the vigilance promoting action of modafinil (Ferraro et al. 1996). This hypothesis is supported by the fact that slow wave sleep in cats are observed upon microinjection of the GABA_A agonist muscimol into the PH (Lin et al. 1989; Sallanon et al. 1989), while Nitz and Siegel (1996) have shown that slow wave sleep is accompanied by an increase in PH extracellular GABA levels. More recently, Azuma et al. (1996) have demonstrated an increase in extracellular glutamate levels in the MPA during wakefulness in freely behaving rats and this finding is in line with a recent report indicating that in the dose range of vigilance enhancement (30–100 mg/kg) modafinil increases glutamate transmission in the ventromedial and ventrolateral thalamus, two subregions of the thalamus involved in the regulation of behavioural arousal (Ferraro et al. 1997a; Mancina and Marini 1997).

In view of the above findings, the aim of the present study was to investigate the effect of vigilance enhancing doses of acute modafinil on extracellular glutamate and GABA levels in the MPA and PH and a possible interaction between these neurotransmitters in these two sleep related brain regions. Towards this aim, we employed dual probe microdialysis to study the effect of intraperitoneal administration of modafinil on extracellular glutamate and GABA levels in the MPA and PH of the intact conscious rat and the effects of local perfusion with a GABA_A receptor agonist or antagonist alone and in the case of the antagonist also with modafinil on extracellular glutamate levels in these regions.

METHODS

Animals

Male adult Sprague-Dawley rats with a body weight of 300–350 g were housed in cages in groups of five ani-

mals at a constant room temperature (20°C) and exposed to a 12:12 h light-dark cycle (lights on at 06.00 a.m.). Food and water were provided ad libitum. Following delivery, the animals were allowed to adapt to the environment for at least one week before the experiment started.

Surgery

On the day of the surgery, the animals were anaesthetised with halothane/air (1.5% mixture) and mounted in a David Kopf stereotaxic frame. The dura was exposed through two holes in the skull and two microdialysis probes of concentric design (outer diameter 0.5 mm; length of dialysing membrane 2 mm), were implanted into the right or the left MPA and the other into the contralateral PH. The coordinates relative to bregma were: MPA = A: –.3; L: .5; V: –9.5; PH = A: –3.6; L: .4; V: –9.0 (Paxinos and Watson 1986). Both cannulae were permanently secured to the skull with stainless steel screws and methacrylic cement. All experiments were performed in awake freely moving rats 36 hours following probe implantation.

Microdialysis Sampling Experiments

On the day of the sampling experiment the microdialysis probes were perfused with Ringer' solution (in mM: Na⁺ 147; K⁺ 4; Ca⁺⁺ 2.4; Cl⁻ 156; Glucose 2.7) at a constant flow rate of 2 µl/min. The collection of samples started 300 min after the onset of the perfusion to achieve stable dialysis extracellular glutamate levels and perfusates were collected every 20 min. After three stable basal values were obtained, modafinil (30–300 mg/kg, suspended in a 0.5% arabic gum solution) or vehicle or saline were intraperitoneally injected and thereafter six more samples were collected (120 min). In this set of experiments each perfusate sample was divided into two parts with 20 µl being assayed for glutamate and 10 µl for GABA. When required the GABA_A receptor agonist and antagonist, muscimol and bicuculline respectively, were included into the perfusion medium. In a final set of experiments the effect of modafinil (100 mg/kg) was evaluated: a) in the presence of bicuculline (.1 and 1 µM), added into the perfusion medium 120 min before the onset of the collection period; b) in the presence of local perfusion with the glutamate uptake inhibitor *L-trans*-pyrrolidine-2,4-dicarboxylic acid (*L-trans*-PDC; Bridges et al. 1991) at a relatively low concentration (.5 mM; Zuiderwijk et al. 1996; Obrenovitch et al. 1997).

At the end of each experiment the brain was removed from the skull and the position of the probes was carefully verified in 30 µm-thick coronal cryostat sections. Only those animals in which both probes were correctly located were included in this study.

Amino Acid Analysis

Endogenous glutamate was quantified using a high performance liquid chromatography (HPLC)/fluorimetric detection system, including precolumn derivatization *o*-phthalaldehyde reagent and a Chromsep 5 (C18) column. The mobile phase consisted of 0.1 M sodium acetate, 10% methanol and 2.5% tetrahydrofuran, pH 6.5. The limit of detection for glutamate was 30 fmol/sample.

Endogenous GABA was quantified using a high performance liquid chromatography (HPLC)/electrochemical detection system, including a precolumn derivatization with an *o*-phthalaldehyde/*t*-butylthiol reagent and a reverse-phase column (Nucleosil 3; C18). The mobile phase consisted of .15 M sodium acetate, 0.1 mM EDTA, pH 5.4 and 50% acetonitrile. The limit of detection for GABA was 20 fmol/sample.

[³H]glutamate Uptake

[³H]glutamate uptake was measured in triplicates at 20°C in both synaptosomes and slices from the entire hypothalamus. Synaptosomes were obtained according to the method of Loscher et al. (1985) and were suspended in cold Krebs-Tris medium (in mM: NaCl 124; KCl 5; KH₂PO₄ 1.25; MgSO₄ 1.2; Trizma base HCl 35; glucose 10; CaCl₂ 1.2; pH 7.4). Slices 400 μm thick were obtained by cutting the hypothalamus sagittally with the McIlwain tissue chopper. Either synaptosomes (0.03–0.06 mg protein) or slices (2) were preincubated with several modafinil concentrations (1–33 μM) or with the highest concentration of DMSO (.13%) used to dissolve modafinil in 1 ml Krebs-Tris medium for 5 min. The uptake process was initiated by the addition of L-[3-⁴H]glutamate, to give a final concentration of 1.0 μM (specific activity: 1nCi/pmol) and was interrupted after 3 min by dilution with 10 ml of cold (0°C) radioactive Krebs-Tris medium followed by filtration under vacuum on Millipore (DAWP; .65 mm). The filters were washed 3 times with 10 ml cold Krebs-Tris medium and dropped into scintillation vials where the radioactivity was measured after the addition of 10 ml Tritosol (Fricke 1975). Blanks were prepared by incubating the preparation used with [³H]glutamate at 0°C.

Statistical Analysis

Data from individual time points were calculated as a percentage of the mean of the three basal samples prior to treatment and expressed as means ± S.E.M. The significance with regard to each time point was calculated and only the peak effect (maximal response) was reported in the figures. In addition, the area created by the curve, which mainly reflects the duration of the effect under 120 min of experimental period, was calcu-

lated for each animal. The area values (overall effects) were expressed as percentage changes in basal value over time (Δ basal % × time) by using the trapezoidal rule. The statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by the Newman-Keuls test for multiple comparisons. The non-parametric Spearman coefficient was used for correlations.

Drugs

Fresh solution of the following drugs were used: Modafinil (Laboratoire L. Lafon, Maison Alfort, France) was suspended in a .5% arabic gum solution for the *in vivo* study or dissolved in DMSO (0.13%) for the *in vitro* experiments. The vehicle was administered to the respective control groups. Muscimol, (–)bicuculline methochloride (Sigma Chemical Company, St Louis, MO, USA) and L-*trans*-PDC, (Tocris, Cookson, Bristol, U.K.) were dissolved to the desired concentration in Ringer perfusion medium immediately before their local administration. L-[3-⁴H]glutamate was purchased from DuPont/NEN (Boston, MA).

RESULTS

The basal extracellular glutamate levels from MPA and PH amounted to $.492 \pm .06$ μM and $.334 \pm .05$ μM, respectively. Basal GABA extracellular levels from the MPA and PH amounted to 16.8 ± 1.5 nM and 15.11 ± 1.9 nM. The absolute values in the saline and vehicle (0.5% arabic gum solution) treated controls were identical and remained stable throughout the experiment (180 min).

Effects of Modafinil on Extracellular Glutamate Levels

As shown in Fig. 1, modafinil (30–100 mg/kg *i.p.*) dose-dependently increased MPA extracellular glutamate levels. The peak effects (maximal responses) for the 60 and 100 mg/kg doses were reached 100 min after drug administration and were $121 \pm 3\%$ and $149 \pm 7\%$ of basal values, respectively. The higher dose (300 mg/kg *i.p.*) did not further increase MPA extracellular glutamate levels. A similar profile of action was also found by analysing the area under the curve values, which mainly reflects the duration of the effect under the experimental period.

Modafinil (30–60 mg/kg, *i.p.*) had no significant effect on PH extracellular glutamate levels, while the 100 mg/kg dose increased PH extracellular glutamate levels to $141 \pm 8\%$. The modafinil-induced increase in ex-

Medial Preoptic Area

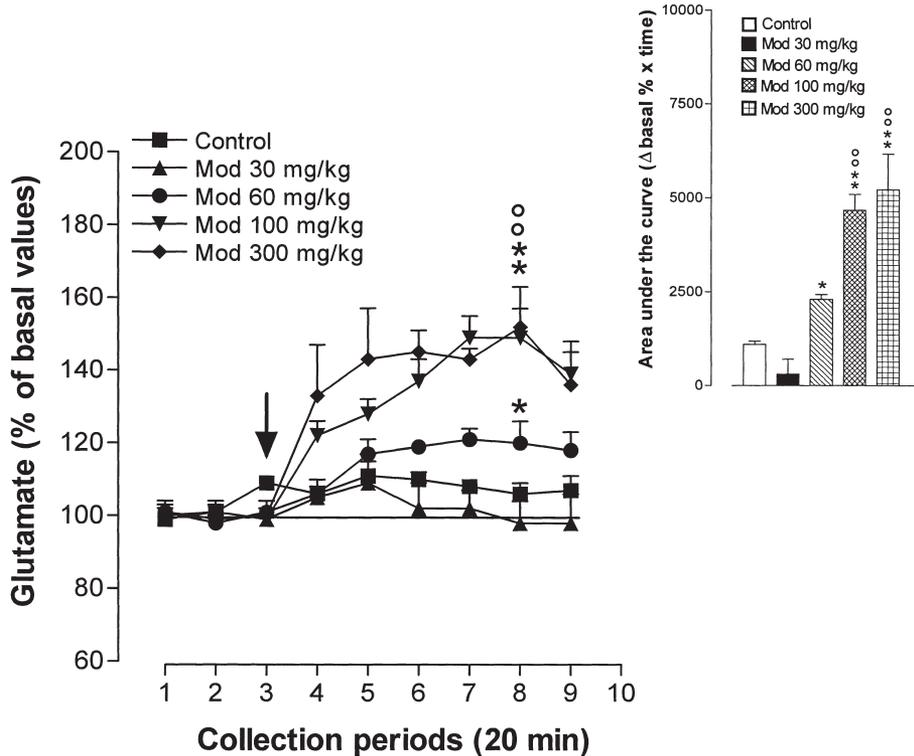


Figure 1. Effects of modafinil (30–300 mg/kg IP) on extracellular glutamate levels from the medial preoptic area of the awake rat. Modafinil or vehicle (0.5% arabic gum solution) were injected at the arrow. Extracellular glutamate levels are expressed as percent of the mean of the three basal values before drug administration (for absolute values, see text). Each point represents the mean ± SEM of five to six animals. The significances for the peak effects are indicated in the figure. The histograms of the areas under the curves, which represent the integrated time-response curve of the effects are shown on the right side of the figure. The areas under the curves were calculated as % changes in basal value over time (Δ basal % \times time) by using the trapezoidal rule. * $p < .05$; ** $p < .01$ significantly different from control as well as modafinil 30 mg/kg; °° $p < .01$ significantly different from modafinil 60 mg/kg according to one-way ANOVA followed by Newman-Keuls test for multiple comparisons.

tracellular glutamate levels at the 300 mg/kg dose was similar to that observed at 100 mg/kg (Fig. 2).

Effects of Modafinil on Extracellular GABA Levels

Interestingly, in the same animals in which glutamate was measured, modafinil in the dose range of 30–100 mg/kg IP dose-dependently decreased extracellular GABA levels in MPA while in the PH it significantly inhibited the extracellular amino acid levels only at 100 mg/kg (Fig. 3). At the higher dose (300 mg/kg) the psychoactive drug did not further decrease MPA and PH extracellular GABA levels.

Effects of Local GABA_A Receptor Activation and Blockade on Extracellular Glutamate Levels in the Medial Preoptic Area and Posterior Hypothalamus

In this set of experiments the effects of local perfusion with the GABA_A receptor agonist muscimol and antagonist bicuculline were studied for their effect on extracellular glutamate levels in the MPA and in the PH. The compounds were included into the perfusion medium 60 min after the onset of sample collection and main-

tained till the end of the experiment. As shown in Fig. 4, local perfusion with muscimol (10 μM) alone reduced extracellular glutamate levels both in the MPA (70 ± 6% of basal values) and the PH (79 ± 4%), while local perfusion with bicuculline (1 μM) alone enhanced MPA (134 ± 6%) and PH (128 ± 8%) extracellular glutamate levels. A similar increase was found when bicuculline was locally perfused at 10 μM concentration both in the MPA (132 ± 7%) and PH (125 ± 5%). At a lower concentration (.1 μM) bicuculline did not affect MPA and PH extracellular glutamate levels (data not shown).

Effects of Local GABA_A Receptor Blockade and Glutamate Uptake Inhibition on the Modafinil-Induced Increase in Extracellular Glutamate Levels

In view of the above results, the effect of the 100 mg/kg dose of modafinil in the MPA and the PH was studied in combination with local perfusion of bicuculline (1 and 0.1 μM). The GABA_A receptor antagonist was included into the perfusion medium 120 min before the onset of sample collection and maintained till the end of the experiment. After three stable basal values were obtained, modafinil (100 mg/kg) was intraperitoneally injected.

Posterior Hypothalamus

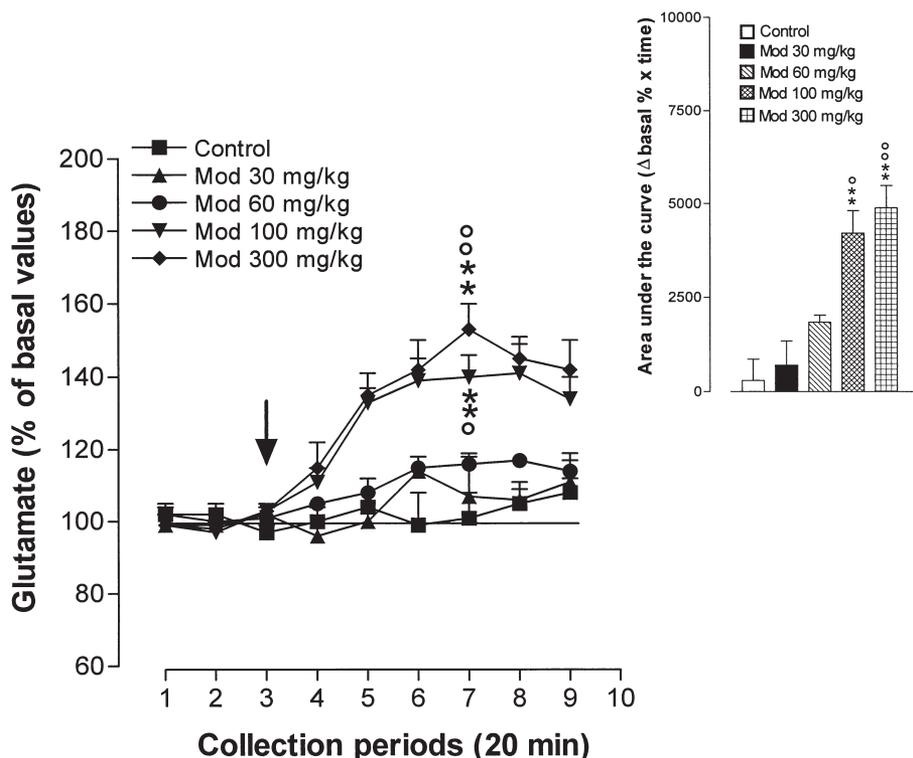


Figure 2. Effects of modafinil (30–300 mg/kg IP) on extracellular glutamate levels from the posterior hypothalamus of the awake rat. Modafinil or vehicle (0.5% arabic gum solution) were injected at the arrow. Extracellular glutamate levels are expressed as percent of the mean of the three basal values before drug administration (for absolute values, see text). Each point represents the mean \pm SEM of five to six animals. The significances for the peak effects are indicated in the figure. The histograms of the areas under the curves, which represent the integrated time-response curve of the effects are shown on the right side of the figure. The areas under the curves were calculated as % changes in basal value over time (Δ basal % \times time) by using the trapezoidal rule. ** $p < .01$ significantly different from control as well as modafinil 30 mg/kg; $^{\circ}p < .05$; $^{\circ\circ}p < .01$ significantly different from modafinil 60 mg/kg according to one-way ANOVA followed by Newman-Keuls test for multiple comparisons.

As expected, in the rats perfused with bicuculline (1 μ M), the basal MPA and PH extracellular glutamate levels were significantly higher ($p < .01$) than that observed in the respective control and bicuculline (0.1 μ M) groups: MPA: control = $.502 \pm .07 \mu$ M; bicuculline (.1 μ M) = $.465 \pm .05 \mu$ M; bicuculline (1 μ M) = $.703 \pm .08 \mu$ M; PH: control = $.321 \pm .05 \mu$ M; bicuculline (.1 μ M) = $.345 \pm .06 \mu$ M; bicuculline (1 μ M) = $.440 \pm .08 \mu$ M. However, in all groups of animals, the basal extracellular glutamate levels remained stable throughout the collection period (180 min) (data not shown). As shown in Fig. 5, the increase in MPA and PH extracellular glutamate levels induced by modafinil alone, was fully prevented in the presence of 1 μ M concentration of bicuculline, while the lower (.1 μ M) concentration did not prevent the modafinil-induced increase of extracellular glutamate levels.

In a final set of experiments in order to demonstrate that this effect was specific to the GABA_A receptor blockade and not related to possibly maximally increased extracellular glutamate levels, the effect of modafinil was also studied in the presence of the glutamate uptake inhibitor *L-trans*-PDC (.5 mM). As shown in Fig. 6, the addition to the perfusion medium of *L-trans*-PDC increased (about +50–60% of the basal values) extracellular glutamate levels both in the MPA and in the PH. Interestingly, when modafinil (100 mg/kg) was injected at the onset of the local perfusion with

L-trans-PDC an additional enhancement of extracellular glutamate levels was found.

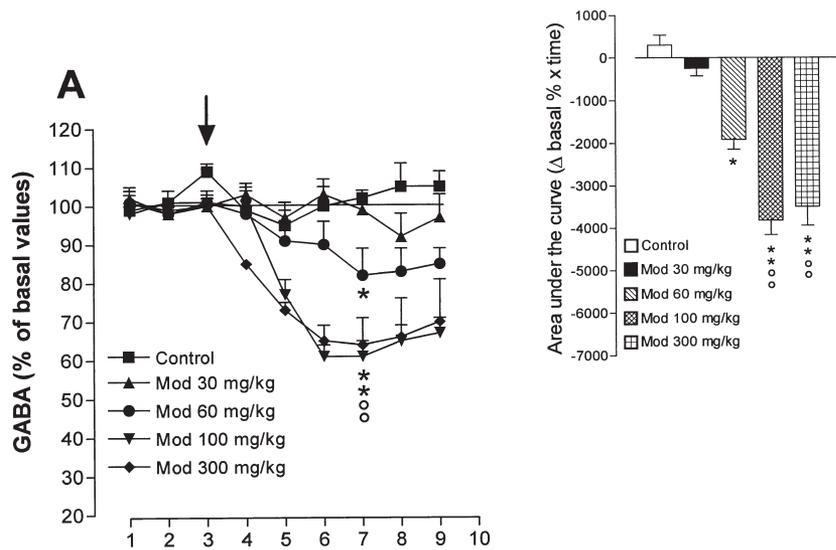
Correlation Analysis of the Modafinil-Induced Changes in Extracellular Glutamate and GABA Levels

The demonstration that the effects of modafinil (100 mg/kg) on extracellular glutamate levels were counteracted by the local application of the GABA_A antagonist bicuculline together with the reduction of MPA and PH extracellular GABA levels induced by modafinil, prompted us to analyse the existence in the same animal of a possible correlation between the effects of modafinil (30–300 mg/kg) on glutamate and GABA extracellular levels, by using the non parametric Spearman test. As shown in Fig. 7, by evaluating the area under the curve values, a significant inverse correlation was found (MPA: $r = -.9752$, $p < .0001$; PH: $r = -.7503$, $p < .0005$).

Effects of Modafinil on the In Vitro Uptake of [³H]glutamate

As shown in Table 1, modafinil (1–33 μ M) failed to affect the uptake of [³H]glutamate both in synaptosomes and in slices from the rat hypothalamus.

Medial Preoptic Area



Posterior Hypothalamus

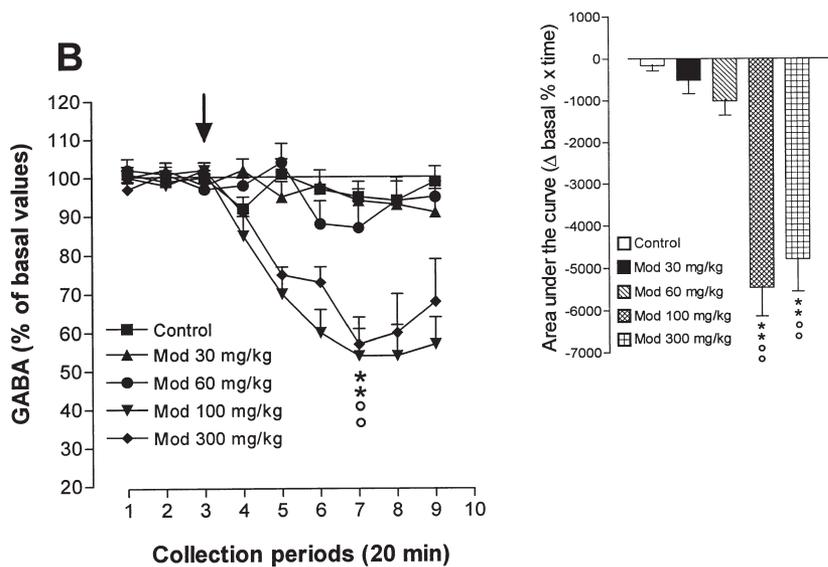


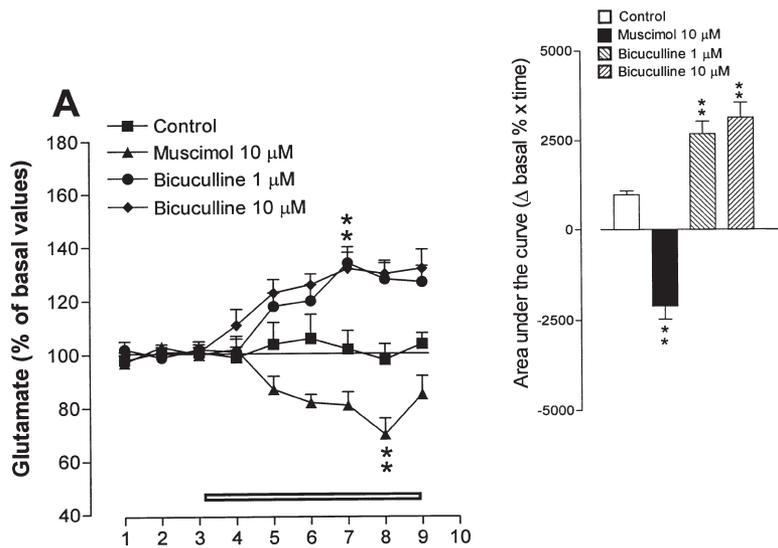
Figure 3. Effects of modafinil (30–300 mg/kg IP) on extracellular GABA levels from the medial preoptic area (panel A) and the posterior hypothalamus (panel B) of the awake rat. Modafinil or vehicle (.5% arabic gum solution) was injected at the arrow. Extracellular GABA levels are expressed as percent of the mean of the three basal values before drug administration (for absolute values, see text). Each point represents the mean \pm SEM of five to six animals. The significances for the peak effects are indicated in the figure. The histograms of the areas under the curves, which represent the integrated time-response curve of the effects are shown on the right sides of the figure. The areas under the curves were calculated as % changes in basal value over time (Δ basal % \times time) by using the trapezoidal rule. * $p < .05$; ** $p < .01$ significantly different from control as well as modafinil 30 mg/kg; $^{\circ\circ}p < .01$ significantly different from modafinil 60 mg/kg according to one-way ANOVA followed by Newman-Keuls test for multiple comparisons.

DISCUSSION

The present microdialysis study provides evidence that modafinil, in vigilance producing doses, increases extracellular glutamate levels probably by a concomitant decrease of extracellular GABA levels (see also Ferraro et al. 1996) in the MPA and PH of the awake freely moving rat, in view inter alia of the significant negative correlation coefficient obtained by measuring simultaneously in the same animal the extracellular levels of both amino acids. In addition, our results demonstrate, for the first time, that in both hypothalamic subregions

the GABA_A receptor agonist muscimol and antagonist bicuculline inhibits and enhances extracellular glutamate levels respectively suggesting that an inhibitory GABAergic tone within the MPA and the PH controls local glutamatergic transmission. A further observation, in line with the existence of an inhibitor GABA/glutamate coupling in the MPA and the PH, is the demonstration that the enhancement of extracellular glutamate levels by modafinil is blocked by prior pre-treatment with bicuculline. The inhibitory action of bicuculline on modafinil-induced increase of MPA and PH extracellular glutamate levels seems to be due to a specific GABA_A

Medial Preoptic Area



Posterior Hypothalamus

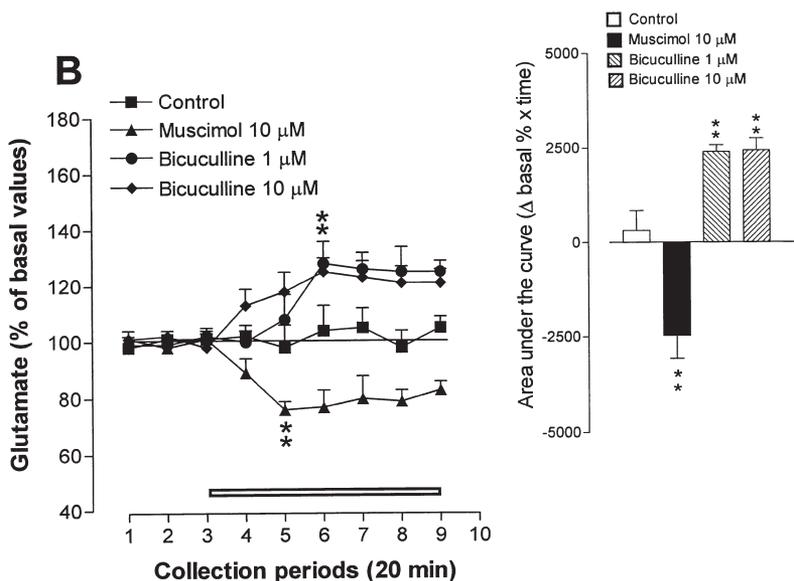
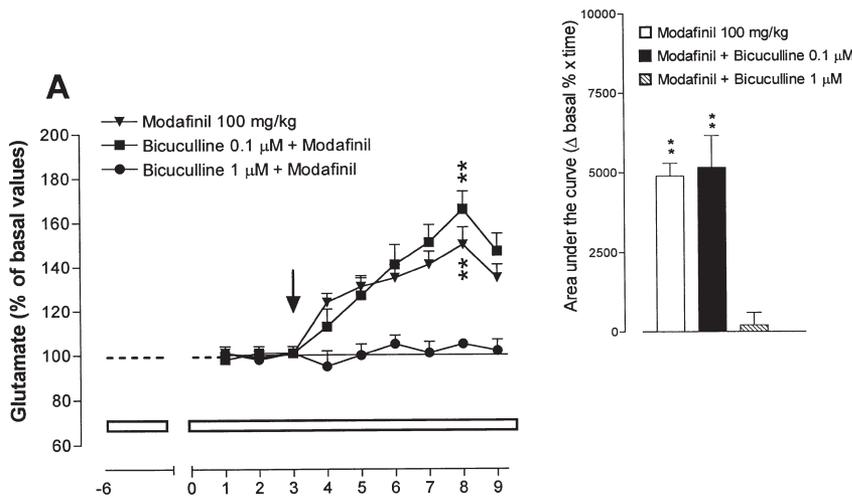


Figure 4. Effects of local perfusion with muscimol (10 μ M) or bicuculline (1 and 10 μ M) on extracellular glutamate levels from the medial preoptic area (panel A) and the posterior hypothalamus (panel B) of the awake rat. The GABA_A receptor agonist or antagonist were included into the perfusion medium 60 min after the onset of sample collection and maintained till the end of the experiment (open bars). Extracellular glutamate levels are expressed as percent of the mean of the three basal values before drug perfusion (for absolute values, see text). Each point represents the mean \pm SEM of five animals. The significances for the peak effects are indicated in the figure. The histograms of the areas under the curves, which represent the integrated time-response curve of the effects are shown on the right side of the figure. The areas under the curves were calculated as % changes in basal value over time (Δ basal % \times time) by using the trapezoidal rule. ** $p < .01$ significantly different from control according to one-way ANOVA followed by Newman-Keuls test for multiple comparisons.

receptor blockade and not to the inability of modafinil to further increase the extracellular glutamate levels already increased by the local application of the GABA_A antagonist. In fact, modafinil (100 mg/kg) further increased extracellular glutamate levels even when the extracellular glutamate levels both in the MPA and in the PH were increased (i.e., +50–60% of the basal levels) by the local perfusion with the highly selective and potent glutamate uptake inhibitor *L-trans*-PDC (Bridges et al. 1991; Mitrovic and Johnston 1994; Obrenovitch et al. 1997). In addition, evidence that the increase in extra-

cellular glutamate levels induced by bicuculline (1 and 10 μ M) (i.e., +30–40% of the basal value) are not maximal, is provided from the findings of previous studies showing that higher increases in MPA and PH glutamate levels may be elicited using a similar methodological approach (Kendrick et al. 1992; Singewald et al. 1995). Taken together, these findings suggest that when the GABA_A receptor in the MPA and PH are fully blocked by bicuculline (1 μ M), as demonstrated by the inability of higher (i.e., 10 μ M) concentrations of bicuculline to further increase local glutamate levels, the inhibitory GABAergic

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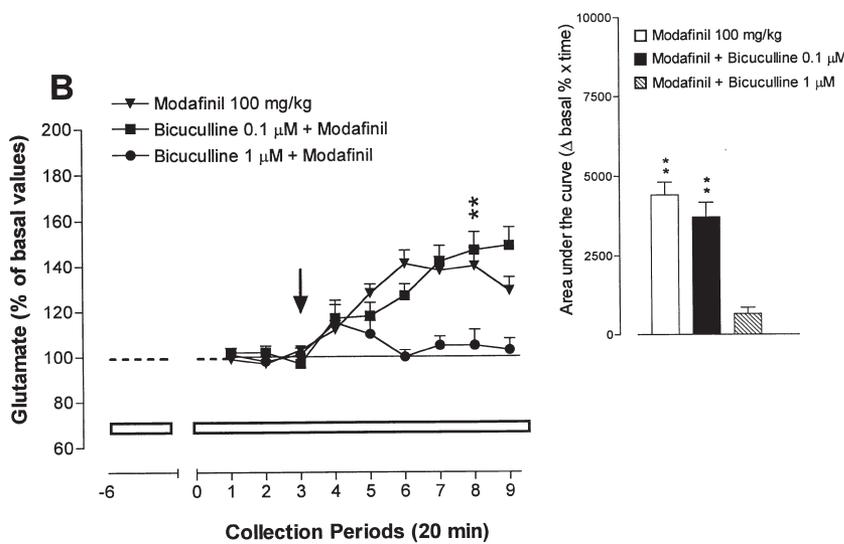


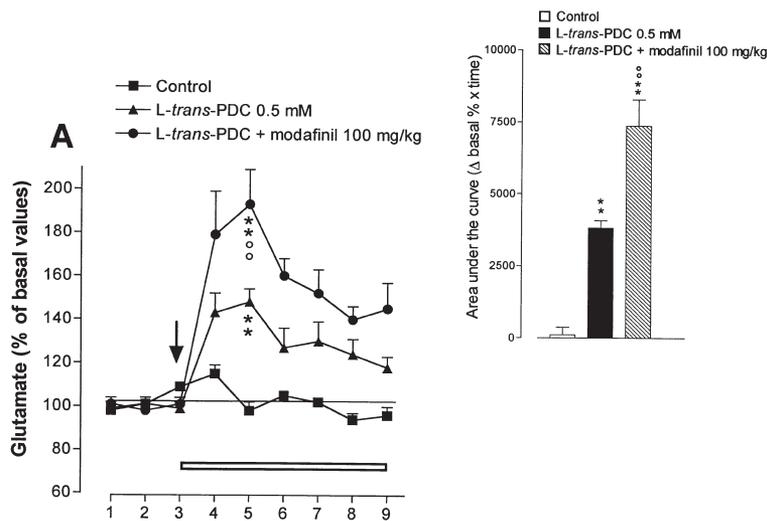
Figure 5. Effects of modafinil (100 mg/kg IP) alone or in combination with the local perfusion of bicuculline (0.1 and 1 μM) on extracellular glutamate levels from the medial preoptic area (panel A) and the posterior hypothalamus (panel B) of the awake rat. The GABA_A receptor antagonist was included into the perfusion medium 120 min before the onset of sample collection and maintained till the end of the experiment (open bars). After three stable basal values were obtained, modafinil (100 mg/kg) was intraperitoneally injected (arrow). Extracellular glutamate levels are expressed as percent of the mean of the three basal values before modafinil administration. Basal extracellular glutamate levels were: MPA: control = .502 ± .07 μM; bicuculline (.1 μM) = .465 ± .05 μM; bicuculline (1 μM) = .703 ± .08 μM; PH: control = .321 ± .05 μM; bicuculline (.1 μM) = .345 ± .06 μM; bicuculline (1 μM) = .440 ± .08 μM. Each point represents the mean ± SEM of five to seven animals. The significances for the peak effects are indicated in the figure. The histograms of the areas under the curves are shown on the right sides of the figure. The areas under the curves were calculated as % changes in basal value over time (Δ basal % × time) by using the trapezoidal rule. ***p* < .01 significantly different from modafinil + bicuculline (1 μM) according to a single one-way ANOVA followed by Newman-Keuls test for multiple comparisons.

tone over the GABA_A receptor is lost and modafinil is unable to further enhance local glutamate levels. Although the effects of the psychoactive drug on GABA and glutamate levels are comparable in the two hypothalamic nuclei, they seem to be region-specific since modafinil (100 mg/kg) failed to affect GABA levels in the hippocampus as well as glutamate levels in the basal ganglia (Ferraro et al. 1997a; Ferraro et al. 1998).

The possibility that modafinil modifies the MPA and PH extracellular glutamate and GABA levels by an effect on their transport into presynaptic nerve terminals or into glial processes, or by an effect on their synthesis seems unlikely. In fact, modafinil does not affect the synthesis of these neurotransmitters *in vitro* (Perez de la Mora et al. 1998) and failed to affect also the [³H]glutamate

uptake into synaptosomes and into slices of the rat hypothalamus (Table 1) and the uptake of [³H]GABA from cortical slices (Tanganelli et al. 1995). In line with these results, modafinil produced an additional increase in extracellular glutamate levels when given at the onset of perfusion with the glutamate uptake inhibitor *L-trans*-PDC. Thus it is reasonable to assume that the modafinil-induced changes in the extracellular levels of glutamate and GABA reported in this study arise as a consequence of an indirect action of modafinil on local hypothalamic neuronal circuits and represents a modification of a nerve impulse activity dependent release process. Since a previous study demonstrated that the 5-HT₃ antagonist MDL 72222, alone and in combination with the nonspecific 5-HT antagonist methysergide,

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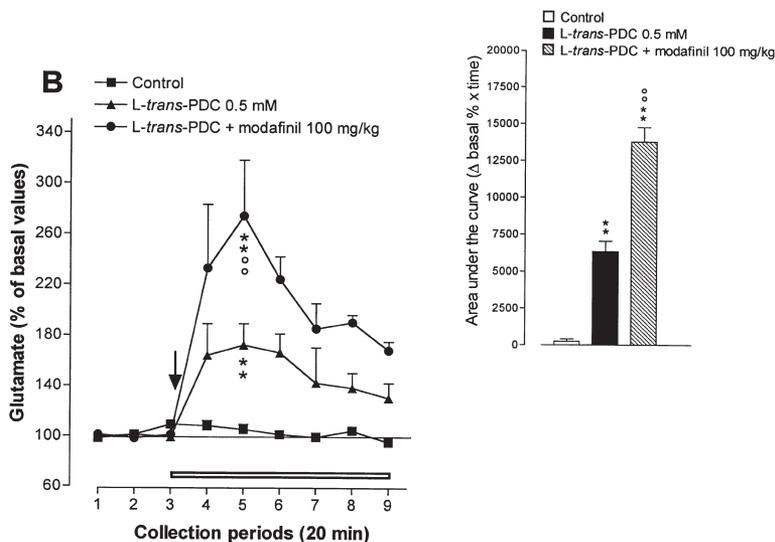


Figure 6. Effects of modafinil (100 mg/kg IP) and L-trans-PDC (0.5 mM), alone or in combination, on extracellular glutamate levels from the medial preoptic area (panel A) and the posterior hypothalamus (panel B) of the awake rat. The glutamate uptake inhibitor was included into the perfusion medium 60 min after the onset of sample collection and maintained till the end of the experiment (open bars) while modafinil was intraperitoneally injected (arrow) at the same time. Extracellular glutamate levels are expressed as percent of the mean of the three basal values before modafinil administration (for absolute values, see text). The significances for the peak effects are indicated in the figure. The histograms of the areas under the curves are shown on the right sides of the figure. The areas under the curves were calculated as % changes in basal value over time (Δ basal % \times time) by using the trapezoidal rule. ** $p < .01$ significantly different from control; $^{\circ}p < .01$ significantly different from L-trans-PDC alone, according to a single one-way ANOVA followed by Newman-Keuls test for multiple comparisons.

substantially counteracts the modafinil-induced inhibition of MPA and PH extracellular GABA levels (Ferraro et al. 1996), it is possible that modafinil indirectly regulates local glutamate and GABA release in these brain regions possibly *via* direct actions on serotonergic mechanism.

Taken together, these findings support the hypothesis that the neurochemical mechanism underlying the vigilance enhancing effects of modafinil may be due, at least in part, to a reduction in local GABAergic inhibitory tone, allowing excitatory glutamate transmission to predominate and to activate key neuronal networks of the preoptic and posterior hypothalamic nuclei which

regulate the sleep/wakefulness cycle. In this context, previous studies suggested that glutamate participates in the regulation of neuronal activity in the MPA which may in turn influence the state of vigilance, e.g. elevated extracellular glutamate levels have been reported in long-sleep mice (Disbrow and Ruth 1984) and an increased glutamate receptor number has been observed in rat forebrain during wakefulness than during sleep (Tononi and Pompeiano 1990). Furthermore, Azuma et al. (1996) using the microdialysis technique, demonstrated that glutamate transmission in the MPA is dynamically involved in the alterations of the vigilance states. It is also worth noting that PH extracellular

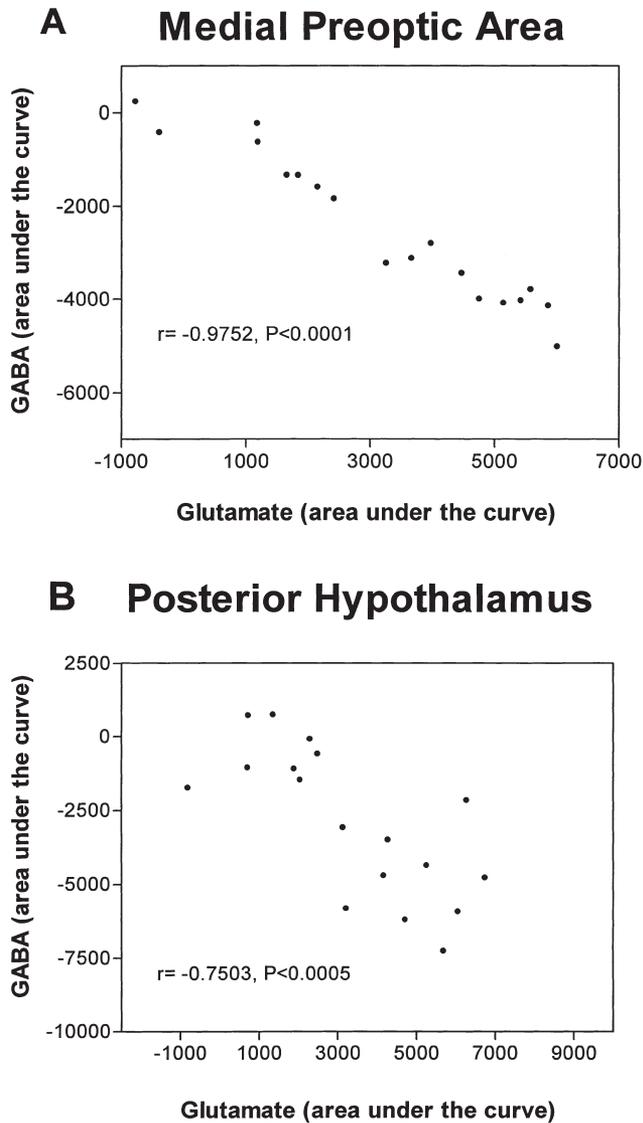


Figure 7. Inverse correlation between changes (as evaluated by the areas under the curves) in extracellular glutamate and GABA levels from the medial preoptic area (A) and the posterior hypothalamus (B) of the awake behaving rat, following modafinil treatments (30–100 mg/kg). The Spearman's correlation coefficient was used.

GABA levels are increased during sleep (Nitz and Siegel 1996) and that the injection of GABA agonist into the tubero-mammillary nucleus, a posterior hypothalamic cell group thought to participate in the modulation of arousal, has been shown to restore sleep in preoptic lesioned, otherwise insomniac, cats (Lin et al. 1989). Finally, electrical stimulation of preoptic area sites in the horizontal hypothalamic slice preparation is associated with GABA_A mediated fast inhibitory postsynaptic potentials in histaminergic neurons (Yang and Hatton 1994).

In conclusion, the present findings suggest that the vigilance enhancing effects of modafinil are associated

Table 1. Effects of Modafinil (1–33 μ M) on [³H]glutamate Uptake by Slices and Synaptosomes of Rat Hypothalamus

Modafinil Concentration (μ M)	Slices (pmol/60 min)	Synaptosomes (pmol/min/mg protein)
Solvent	93 \pm 6	220 \pm 13
1.0	84 \pm 7	220 \pm 26
3.3	84 \pm 3	203 \pm 20
10.0	92 \pm 10	220 \pm 13
33.0	95 \pm 10	223 \pm 16

The results are expressed as means \pm SEM of 5 different experiments. The uptake in slices was measured in 2 slices; .03–.06 mg were used for the synaptosomal uptake. For details, see Methods.

with a decrease in GABA and an increase in extracellular glutamate levels in the MPA and PH. The enhanced glutamate drive may activate networks in the MPA and PH mediating behavioural and EEG arousal. These findings may be of importance in elucidating the mechanism of action of modafinil and in determining the role of the MPA and PH in behavioural and EEG arousal.

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