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Modafinil and cortical γ-aminobutyric acid outflow. Modulation by 5-hydroxytryptamine neurotoxins

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Abstract

The acute or chronic administration of modafinil, (diphenyl-methyl-sulfinyl-2-acetamide, 30 mg/kg s.c.) decreased γ-aminobutyric acid (GABA) outflow from the cerebral cortex of freely moving guinea pigs and rats. In 5,7-dihydroxytryptamine intracerebroventricularly pretreated guinea pigs, the effect of modafinil on GABA outflow was reversed and the noradrenaline cortical levels increased. Prazosin (35,8 ng/kg i.p.) blocked the drug-induced increase in GABA efflux. In vitro experiments, performed in rat cortical slices, showed that modafinil failed to affect [^{3}H]GABA release and uptake as well as glutamic acid decarboxylase activity. In conclusion, our results suggest that the balance between central noradrenaline and 5-hydroxytryptamine transmission is important for the regulation by modafinil of the GABAergic release in the cerebral cortex.

Keywords: GABA (γ-aminobutyric acid); Release; Epidural cup; Modafinil; 5-HT (5-hydroxytryptamine, serotonin); Cerebral cortex

1. Introduction

Evidence obtained from previous studies indicates that the psychoactive drug, modafinil, (diphenyl-methyl)-sulfinyl-2-acetamide (Hermant et al., 1991), acutely inhibits the activity of cortical γ-aminobutyric acid (GABA) neurons (Tanganelli et al., 1992). Such an inhibition still persists, although less long-lasting, after a 7-day treatment with the drug. The modafinil-induced inhibition is prevented by the 5-HT$_2$ receptor antagonist ketanserine, as well as by i.c.v. 6-hydroxy-dopamine pretreatment (Tanganelli et al., 1994). These results suggest that the serotonergic and noradrenergic tone determines the action of modafinil on cortical GABA release in the awake guinea pig. In the present study, this analysis was continued by evaluating the effects of the drug in the 5,7-dihydroxytryptamine (5,7-DHT) treated awake guinea pig. In addition, in order to ascertain whether GABA inhibition could be elicited also in vitro, the influence of modafinil on GABA release, uptake and synthesis was investigated in neocortical and hippocampal slices.

2. Material and methods

2.1. In vivo experiments

Guinea pigs of both sexes (400–450 g) and male specific pathogen-free Wistar rats (200–250 g) were used. The animals, housed in a temperature-controlled environment, were kept on a 12 h ligh/dark cycle, and had free access to water and standard laboratory food.

2.1.1. Surgical procedure

Eight days before the release experiment the animals were anesthetized by free breathing of a 1.5-98.5% mixture of halothane and oxygen. The guinea pigs and rats were mounted into a stereotaxic frame and an

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intracerebroventricular stainless guide cannula was permanently implanted to allow the i.c.v. administration of 5,7-dihydroxytryptamine (5,7-DHT) or vehicle phosphate buffered saline (PBS). Two days before the release experiment, each animal was again anesthetized with an intraperitoneal injection of ketamine (100 mg/kg) and on the contralateral side to the cannula (right parietal cortex) an epidural cup (perspex cylinder 4 mm inner diameter; 0.5 ml capacity) was screwed, using a procedure previously described in detail (Beani et al., 1968, Tanganelli et al., 1992). During the surgical procedure great care was taken to avoid damaging the underlying dura mater and full sterile precautions were maintained during and after the implantations.

2.1.2. Treatments of guinea pigs

Modafinil. On the day of the implantation of the i.c.v. cannula the guinea pigs were treated with modafinil 30 mg/kg s.c. or with the vehicle (arabic gum 0.5%). These treatments were repeated every morning at the same time for 8 days up to the day of the release experiment.

5,7-Dihydroxytryptamine (5,7-DHT). Twenty-four hours after the implantation of the cannula, both groups of animals [previously treated (1 h) with desmethylimipramine 25 mg/kg i.p., to block noradrenaline uptake] were injected i.c.v., with 125 μg of 5,7-DHT (dissolved in a 10 μl volume of PBS solution) or with PBS alone (Bianchi et al., 1986). When required, the α_1-adrenoreceptors were blocked with prazosin at a selective and low i.p. dose of 35.8 ng/kg (see Beani et al. 1988).

2.1.3. Experimental design of cortical GABA release

To study the efflux of endogenous GABA the cup was filled with 0.4 ml of Ringer solution (composition in millimolar: NaCl, 155; KCl, 5,6; $CaCl_2$, 2.2; $MgCl_2$, 0.52; and glucose, 2.7) containing 2 mM of ethanolamine-O-sulphate (an irreversible inhibitor of GABA metabolism, for details see Tanganelli et al., 1992). On the day of the release experiment at least four samples were collected every 30 min to measure basal GABA efflux in 5,7-DHT-treated and untreated animals. Thereafter, modafinil 30 mg/kg or gum arabic was injected subcutaneously and four subsequent samples were collected. Thus, the effect of the psychoactive drug was evaluated either in guinea pigs and rats that received modafinil (acute groups) for the first time or in guinea pigs treated with modafinil for 8 days (chronic groups). The animals receiving gum arabic were designated as controls. The experimental protocol is summarized in Table 1.

2.1.4. GABA assay

GABA outflow was measured by a mass-fragmentographic technique using a selective multiple ion detection method. Details of the analysis have already been given (Bertilsson and Costa, 1976; Moroni et al., 1981).

2.1.5. Monoamine levels in the cortex of guinea pigs

At the end of each release experiment the guinea pigs were killed by decapitation, the brains quickly removed and the right cortex under the cup dissected out on ice and stored at −80°C. The content of noradrenaline, dopamine, 5-HT (serotonin, 5-hydroxytryptamine) and their metabolites was measured using high performance liquid chromatography (HPLC) with electrochemical detection (for details see Keller et al., 1976; Fuxe et al., 1992).

2.2. In vitro experiments

Male specific pathogen-free Wistar rats (200–250 g) were used.

Table 1
Schematic representation of in vivo treatments

Group	Day					
	1 Cannula implantation	2	3–6	7 Epidural cup implantation	8	9 Release experiment
Control	Gum arabic	Gum arabic PBS (i.c.v.)	Gum arabic	Gum arabic	Gum arabic	Gum arabic
Acute	Gum arabic	Gum arabic PBS (i.c.v.)	Gum arabic	Gum arabic	Gum arabic	Modafinil
Chronic	Modafinil	Modafinil PBS (i.c.v.)	Modafinil	Modafinil	Modafinil	Modafinil
Control 5,7-DHT	Gum arabic	Gum arabic 5,7-DHT (i.c.v.)	Gum arabic	Gum arabic	Gum arabic	Gum arabic
Acute 5,7-DHT	Gum arabic	Gum arabic 5,7-DHT (i.c.v.)	Gum arabic	Gum arabic	Gum arabic	Modafinil
Chronic 5,7-DHT	Modafinil	Modafinil 5,7-DHT (i.c.v.)	Modafinil	Modafinil	Modafinil	Modafinil

2.2.1. Brain slices

In order to study [^{3}H]GABA release and uptake, slices of the frontoparietal cortex were obtained from an isolated block of this cortex. The tissue was sliced with a McIlwain tissue chopper, with the blade oriented perpendicularly to the longitudinal axis of the cortex in order to keep intact the laminar anatomical organization of the cortex. The resulting slices were 300 μm thick and weighed 1.1 mg each as an average. Dorsal hippocampal slices were obtained from a piece of this region in the same way as from the cortex.

2.2.2. [^{3}H]GABA release

[^{3}H]GABA release was measured essentially as described by Pérez de la Mora et al. (1993) but with some modifications. Namely, brain slices were incubated at 37°C for 15 min in 1.0 ml of a Krebs-Ringer medium (118 mM NaCl, 1.2 mM KH_2PO_4, 4.7 mM KCl, 1.17 mM $MgSO_4$, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, and 5.6 mM glucose, pH 7.4) gassed with a mixture of 95% O_2, 5% CO_2 and containing 1 μM [^{3}H]GABA (20 Ci/mmol). At the end of this uptake period the slices were transferred to 0.25 ml chambers (4–5 slices per chamber) and were superfused at 0.5 ml/min with fresh Krebs-Ringer medium for 50 min to obtain a constant release of [^{3}H]GABA (basal release). Modafinil in 5% dimethyl sulfoxide or a corresponding amount of 5% dimethyl sulfoxide was added to the media of the experimental and control chambers, respectively, to give a final concentration of 1.0 μM modafinil and 0.0011% dimethyl sulfoxide and the superfusion was continued for a further 10 min in order to evaluate the effect of modafinil on the basal release of [^{3}H]GABA. At the end of this period, the slices were stimulated for 2 min with 30 mM KCl and the superfusion was continued as before. In order to prevent the breakdown of [^{3}H]GABA, 50 μM aminooxyacetic acid was present during [^{3}H]GABA uptake and during the whole superfusion. Fractions were collected every min and at the end of the experiment the slices were digested in 0.5 ml 1.0% sodium dodecyl sulphate. The radioactivity in both slices and fractions was counted by scintillation spectrometry in vials containing 5.0 ml Tritosol (Fricke, 1975). Since we have shown (Pérez de la Mora et al. 1993) that [^{3}H]GABA accounts for 90% of the radioactivity released under the present superfusion conditions and for 80% of the radioactivity stored in the slices at the end of the superfusion, we will refer to the radioactivity stored within the tissue or released by the slices as [^{3}H]GABA. [^{3}H]GABA release was calculated in each fraction as the percent of the [^{3}H]GABA present in the tissue at the beginning of the collection of fractions. In order to evaluate the effects of modafinil on the basal release of [^{3}H]GABA, the average of the [^{3}H]GABA released in the three fractions collected before and in the three fractions collected after the introduction of modafinil was calculated, and the percent of [^{3}H]GABA released in the presence of modafinil was obtained with respect to the [^{3}H]GABA released in its absence. In order to evaluate the effect of modafinil on the K^+-stimulated release of [^{3}H]GABA, the basal [^{3}H]GABA release was subtracted from the peak fraction containing the K^+-stimulated [^{3}H]GABA release and the percent of the K^+-stimulated [^{3}H]GABA release with respect to the basal [^{3}H]GABA release was obtained. For each experiment, [^{3}H]GABA release was calculated as the average of three different superfusions.

2.2.3. [^{3}H]GABA uptake

The total high-affinity [^{3}H]GABA uptake was studied in slices of the frontoparietal cortex as described by Iversen and Neal (1968) with some modifications. Four slices were preincubated in triplicate (15 min) at 25°C in 1.0 ml of an oxygenated Krebs-Tris medium (124 mM NaCl, 5 mM KCl, 1.25 mM KH_2PO4, 1.2 mM MgSO4, 35 mM tris HCl pH 7.4, 2.0 mM $CaCl_2$, 10 mM glucose), containing 0.01 mM amino-oxyacetic acid. Aliquots, 10 μl, of concentrated modafinil solutions were added to cover a concentration range from 1 to 100 μM and the slices were preincubated for a further 10 min. [^{3}H]GABA (0.2 Ci/mmol) was then added to give a final 1.0 μM concentration and 10 min afterwards the uptake process was stopped by rapid filtration of the media through 2.5 cm Whatman No. 4 paper disks layered on a Millipore manifold. The slices were washed 4 times with 3.0 ml of fresh Krebs-Tris medium adjusted to 25°C and were then transferred together with the paper disks to scintillation vials. [^{3}H]GABA was extracted from the slices into 1.0 ml H_2O by shaking the vials for 1.0 h. Finally, after the addition of 5 ml Tritosol (Fricke, 1975) the radioactivity was estimated. Blanks in which NaCl was substituted for choline chloride were carried along with the samples and radioactivity was subtracted from the experimental samples. Glial [^{3}H]GABA uptake was defined as the residual [^{3}H]GABA accumulation observed when the [^{3}H]GABA uptake was carried out in the presence of 1.0 mM of the specific neuronal [^{3}H]GABA uptake blocker L-2,4-diaminobutyric acid (Iversen and Johnston, 1971; Schon and Kelly, 1974). Control experiments showed that no further inhibition (70%) of [^{3}H]GABA uptake was obtained by using higher L-2,4-diaminobutyric acid concentrations (data not shown). Net GABA uptake (pmol/min per g) was calculated from the specific activity of the [^{3}H]GABA used. The total and glial low affinity [^{3}H]GABA uptake was studied as above but with incubation of the slices with 100 μM [^{3}H]GABA (20 μCi/μmol).

2.2.4. Measurement of glutamic decarboxylase activity

For each experiment the rat brain was homogenized in 5 volumes of ice-cold 60 mM K-phosphate buffer;

6.5 mM dithiothreitol pH 6.4 and a concentrated solution of Triton X-100 was added to give a 0.5% concentration. The homogenate was left in ice for 15 min and then was divided into two portions. One portion was supplemented with pyridoxal phosphate to give a 0.2 mM concentration in the incubation mixture and a corresponding amount of buffer was added to the other portion. The glutamic acid decarboxylase extracts were finally obtained by centrifuging the homogenate (4°C) at $100000 \times g$ for 30 min. Modafinil was added to different portions of the extracts to cover a concentration range from 1 to 1000 μM and 5.0 min after the enzyme activity was measured. Glutamic decarboxylase activity was assayed in triplicate as described by Pérez de la Mora et al. (1992) using a reaction device similar to that described by Albers and Brady (1959). Protein content was measured according to Lowry et al. (1951).

2.3. Statistical analysis

The results of the in vivo release experiments are reported as percent changes from the mean of the three basal values before treatment and as the area created by the curve. The area values (overall effects) were expressed as percentage changes in arbitrary units and were calculated for each animal. The data on monoamine levels were expressed in absolute values (ng/g wet weight). The statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by the Student Newman-Keuls (SNK) test for multiple comparisons. The non-parametric Dunn's test for the analysis of variance (Hollander and Wolfe, 1973) was used in the in vitro experiments to compare different experimental conditions to each other.

2.4. Drugs

Modafinil (Laboratoire L. Lafon, Maisons Alfort, France) was suspended in a 0.5% gum arabic solution for the in vivo experiments. The same vehicle was injected to the control animals. For the in vitro experiments the drug was dissolved in dimethyl sulfoxide and diluted thereafter with water to give a concentration of 1.9 mg/ml modafinil, 8% dimethyl sulfoxide. Working solutions of modafinil were prepared by serial water dilutions of this last modafinil stock solution. 5,7-Dihy-

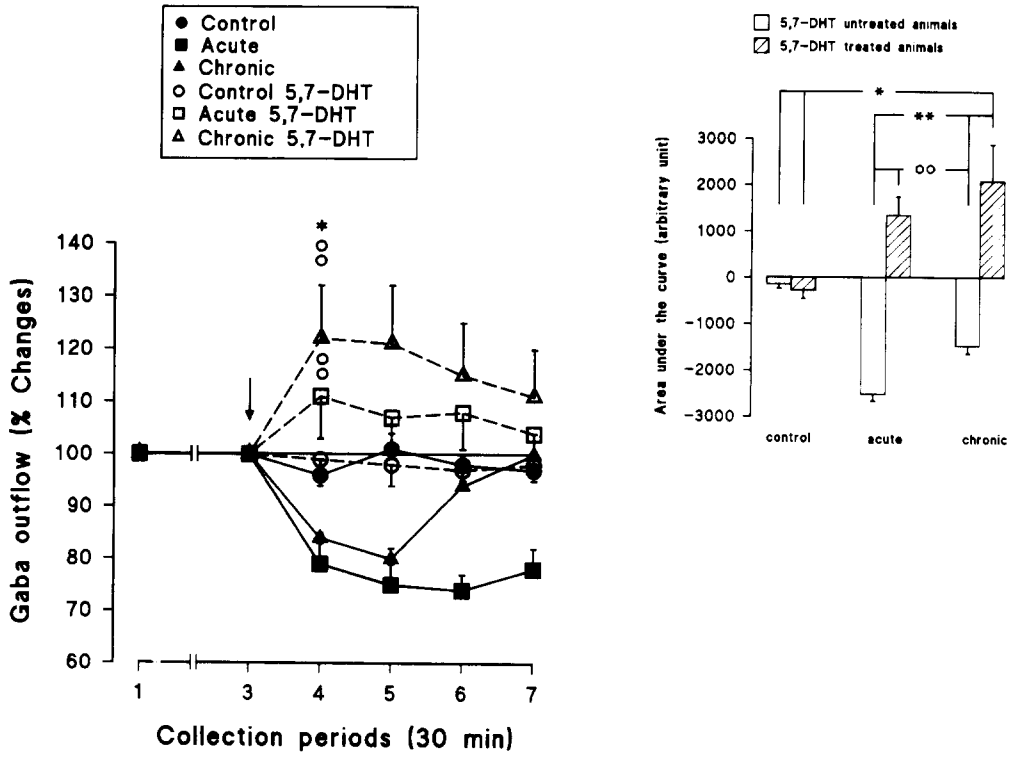

Fig. 1. Effects of acute and chronic administration of modafinil (30 mg/kg s.c.) on the GABA outflow from the parietal cortex of 5,7-DHT-treated and untreated awake freely moving guinea pigs. In the chronic group modafinil was given daily for 8 days while 5,7-DHT was injected i.c.v. (125 μg/10 μl PBS) 7 days before the experiment (for details see Material and methods). Modafinil or the vehicle (control) was injected at the arrow. GABA outflow was expressed as percent of the mean of the three basal values before drug administration (for absolute values, see text). Each point represents the mean ± S.E.M. for six to eight animals. The peak increases of GABA release were statistically evaluated. The histograms of the areas under the curves are shown on the right side of the figure. * $P < 0.05$; ** $P < 0.01$ statistically different from control; $^{\circ\circ}$ $P < 0.01$ statistically different from acute as well as chronic modafinil-treated sham-operated animals (SNK-test for multiple comparisons).

droxytryptamine, amino-oxyacetic acid, L-2,4-diaminobutyric acid (Sigma Chemical Co., St. Louis, Mo, USA); desmethylimipramine (Chiesi, Italy); prazosin (Pfizer, New York, NY, USA); 2,3[^{3}H]GABA (30 Ci/mmol) and L-[1-^{14}C]glutamic acid (75.6 μCi/mmol) (New England Nuclear, California, USA) were used. 5,7-Dihydroxytryptamine was dissolved in a phosphate-buffered saline solution. All solutions were freshly prepared. All other chemicals were obtained from local sources and were of the purest grade available.

3. Results

3.1. In vivo release experiments

3.1.1. Basal GABA outflow in guinea pigs and rats

In control groups, the cortical GABA outflow was constant and no significant variation was observed after the vehicle injection (arabic gum). The mean basal value was 528 ± 24 pmol/cm^2 per 30 min (means ± S.E.M. for 12 animals) with the guinea pigs and 685 ± 45 pmol/cm^2 per 30 min (means ± S.E.M. of 8 animals) with the rats (Figs. 1 and 2).

3.1.2. Effect of acute and chronic modafinil treatment

Animals not treated with 5,7-DHT. As shown in Fig. 1, the permanent implantation of an intracerebroventricular stainless guide cannula and the subsequent injection of PBS, did not affect the profile of the acute and chronic modafinil-induced inhibiton of cortical GABA outflow, previously demonstrated in non-implanted guinea pigs (Tanganelli et al., 1994). The effect of acute modafinil administration (30 mg/kg s.c.) was also tested in male Wistar rats and a rapid and long-lasting inhibition of cortical GABA outflow, similar to that observed in the guinea pigs, was found (Fig. 2).

5,7-DHT-treated guinea pigs. As shown in Fig. 1, the intracerebroventricular injection of 5,7-DHT did not influence the basal (468 ± 47 pmol/cm^2 per 30 min; means ± S.E.M. for 16 animals) cortical GABA outflow with respect to the non-lesioned group. Interestingly, the acute treatment with modafinil (30 mg/kg s.c.) induced, in lesioned animals, a slight increase in GABA release (+15% of basal values) instead of the inhibition observed in control guinea pigs. In the chronic modafinil group (Fig. 1) the drug produced a

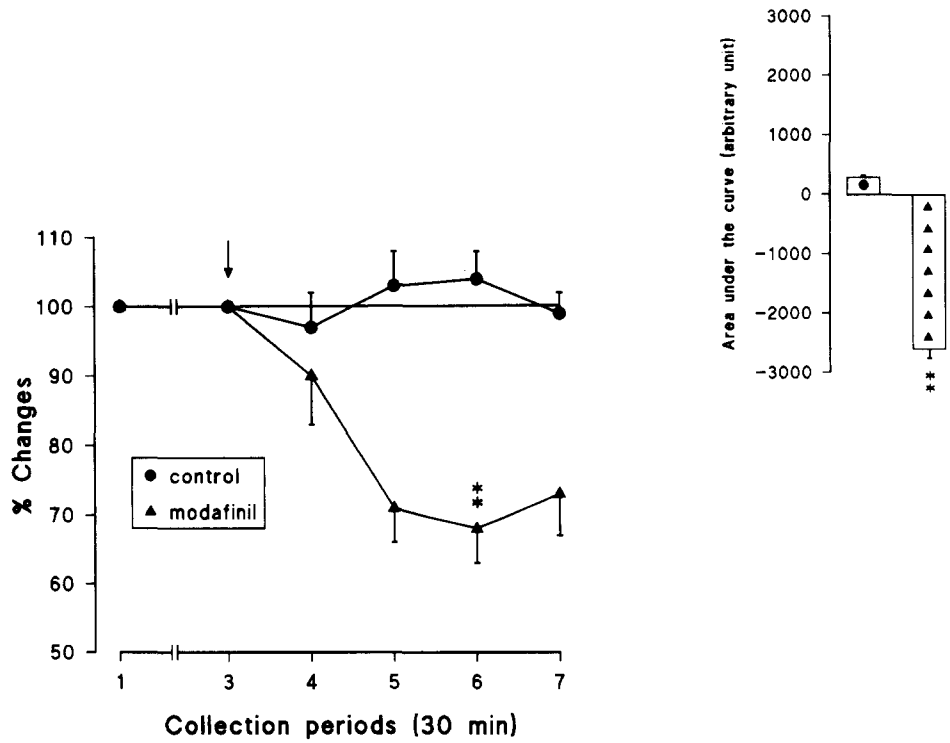

Fig. 2. Effect of acute administration of modafinil (30 mg/kg s.c.) on the GABA outflow from the frontoparietal cortex of awake freely moving rats equipped with an epidural cup. Modafinil or the vehicle (control) was injected at the arrow. GABA outflow was expressed as percent of the mean of the three basal values before drug administration. Each point represents the mean ± S.E.M. for eight animals. The peak reduction was statistically evaluated. The histograms of the areas under the curves are shown on the right side of the figure. ** $P < 0.01$ statistically different from the control group (Student's t-test).

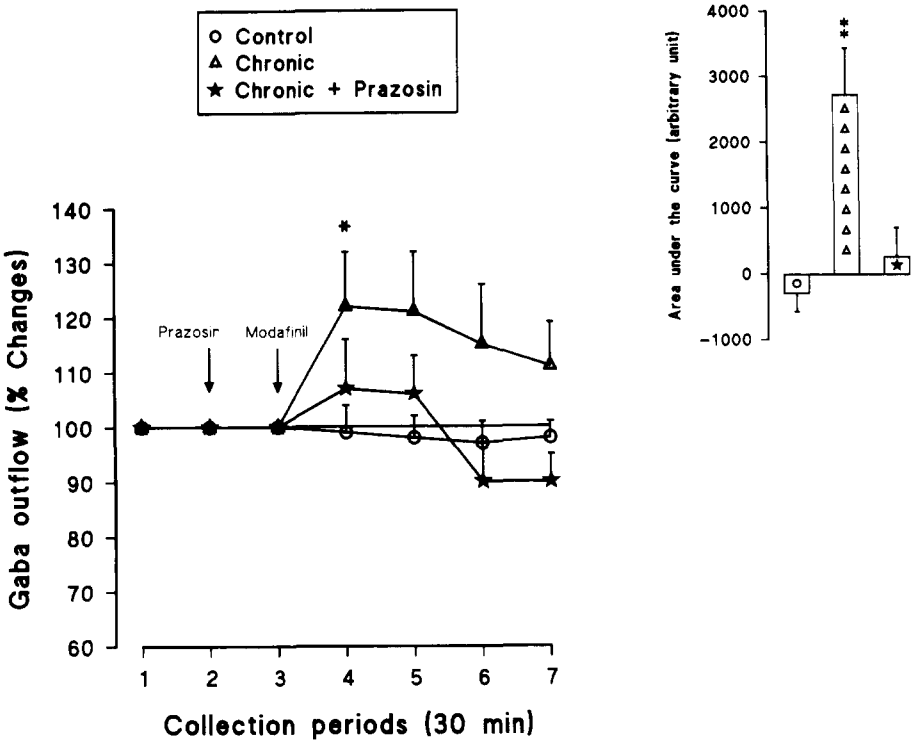

Fig. 3. Effects of chronic administration of modafinil (30 mg/kg s.c.) alone and in the presence of prazosin (35.8 ng/kg i.p.) on the GABA outflow from parietal cortex of 5,7-DHT (125 μg/10 μl PBS i.c.v. 7 days before the experiment) treated awake freely moving guinea pigs. Modafinil was given daily for 8 days before the experiment (for details see Material and methods). GABA outflow was expressed as percent of the mean of the three basal values before drug (for absolute values, see text). Each point represents the mean ± S.E.M. for seven to eight animals. The peak increases of GABA release were statistically evaluated. The histograms of the areas under the curves are shown on the right side of the figure. * $P < 0.05$; ** $P < 0.01$ statistically different from the control group (SNK test for multiple comparisons).

more consistent and prolonged enhancement of GABA outflow, which was significantly different from its lesioned control group. The effect reached a peak (+25% of basal value) 30 min after the injection and the facilitatory action disappeared 60 min later. Interestingly, in the chronic modafinil group the increase of cortical GABA release was completely prevented by the α_1-adrenoceptor antagonist, prazosin (35.8 ng/kg, i.p., a dose itself inactive on GABA release), administered 30 min before modafinil treatment (Fig. 3).

3.1.3. Monoamine levels in the neocortex

5-HT levels. At the end of the release experiment, the monoamine levels within the cortical tissue under the cup were measured in all groups of guinea pigs. As shown in Fig. 4, a single i.c.v. injection of 125 μg of 5,7-DHT, given 7 days before the release experiment, significantly reduced cortical 5-HT levels. Chronic treatment with modafinil (8 days) significantly counteracted the reduction induced by the administration of the toxin and restored the 5-HT levels to normal values.

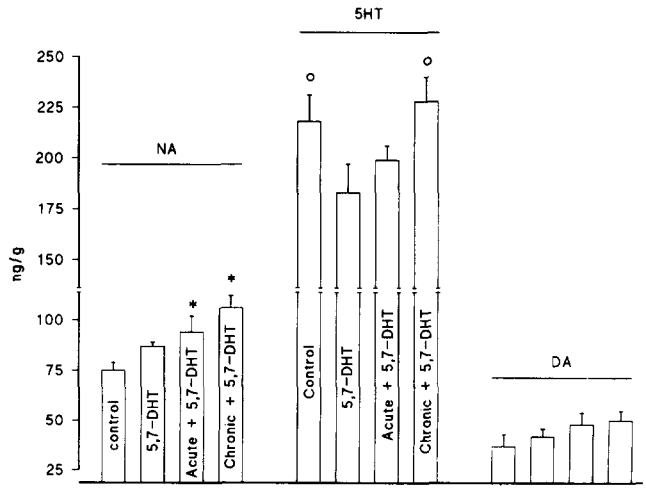

Fig. 4. Effects of acute and chronic administration of modafinil (30 mg/kg s.c.) on NA, 5-HT and DOPAMINE levels in the parietal cortex, under the epidural cup, of 5,7-DHT (125 μg/10 μl PBS i.c.v., 7 days before the experiment)-treated awake freely moving guinea pigs. The repeated modafinil doses were given daily for 8 days before the experiment (for details see Materials and methods). Each value represents the mean ± S.E.M. for six to eight animals. * $P < 0.05$; ** $P < 0.01$ statistically different from control; ° $P < 0.05$ statistically different from 5,7-DHT-treated animals (SNK test for multiple comparisons).

Table 2
Effects of modafinil (1 μM) on the release of [^{3}H]GABA from rat brain slices

Region	[^{3}H]GABA release			
	Basal		K^+-stimulated	
	Control	Modafinil	Control	Modafinil
Frontoparietal cerebral cortex $n = 4$	97 ± 7 (0.16 ± 0.013)	87 ± 6 (0.18 ± 0.1)	500 ± 45 (0.15 ± 0.02)	497 ± 45 (0.14 ± 0.017)
Dorsal hippocampus $n = 11$	81 ± 1.1 (0.16 ± 0.007)	84 ± 4.5 (0.17 ± .01)	485 ± 53 (0.16 ± 0.013)	447 ± 59 (0.12 ± 0.006)

Basal [^{3}H]GABA release is expressed in percent of the [^{3}H]GABA released before the addition of modafinil or dimethylsulfoxide to the superfusion medium. K^+-stimulated [^{3}H]GABA release is expressed in percent of the basal [^{3}H]GABA released before the K^+ stimulation. The data are means ± S.E.M. for the number (n) of experiments indicated. For each experiment [^{3}H]GABA release was calculated as the average of three different superfusions. Absolute values for the [^{3}H]GABA released before the addition of the test compounds are given in parentheses and shown as percent of total tissue [^{3}H]GABA at the beginning of the collection of the fractions. See Materials and methods for further details on the experimental and the calculation procedures.

Table 3
Effects of modafinil on the high-affinity uptake of GABA in slices of the rat frontoparietal cerebral cortex

Concentration of modafinil (μM)	GABA uptake (pmol/min per g)	
	Total	Glial
–	210 ± 12 (5)	120 ± 14 (4)
1	191 ± 14 (5)	97 ± 6 (4)
10	207 ± 8 (5)	112 ± 16 (4)
100	208 ± 23 (5)	120 ± 12 (4)

High-affinity GABA uptake was studied by evaluating the accumulation of 1 μM [^{3}H]GABA (0.2 Ci/mmol) into cortical slices. Glial GABA uptake was obtained by carrying out the whole experimental procedure in the presence of 1 mM L-2,4-diaminobutyric acid. The net GABA uptake (pmol/min per g) was calculated from the specific activity of the [^{3}H]GABA used. Means ± S.E.M. of five independent experiments are shown.

Table 4
Effects of modafinil on the low-affinity uptake of GABA in slices of rat frontoparietal cerebral cortex

Concentration of modafinil (μM)	GABA uptake (nmol/min per g)	
	Total	Glial
–	14.3 ± 1.4	8.0 ± 1.1
1	14.8 ± 1.4	9.8 ± 1.7
10	14.9 ± 1.6	8.3 ± 1.6
100	13.9 ± 1.7	8.0 ± 0.8

The low-affinity uptake of GABA was studied by evaluating the accumulation of 100 μM [^{3}H]GABA (20 Ci/μmol) into cortical slices. The net GABA uptake (nmol/min per g) was calculated from the specific activity of the [^{3}H]GABA used. Means ± S.E.M. of five independent experiments are indicated. For a complete description of the experimental procedure see Materials and methods.

Catecholamine levels. 5,7-DHT treatment itself induced a slight but non-significant increase of noradrenaline levels. This enhancement, however, became significant ($P < 0.05$) when the guinea pigs were treated with 5,7-DHT plus modafinil either acutely or chronically and in this last group the increase became more consistent. Conversely, dopamine levels were not affected by the treatment with either 5,7-DHT alone or in combination with acute or chronic modafinil treatment (Fig. 4).

3.2. *In vitro experiments*

All the in vitro experiments were carried out with rat brain slices.

3.2.1. *Effects of modafinil on the release of [^{3}H]GABA*

To ascertain whether modafinil elicited its inhibitory effects on the GABA outflow by directly affecting the GABAergic structures, the effects of this drug were studied on the release of [^{3}H]GABA from slices of the frontoparietal cortex and the dorsal hippocampus of the rat. No effect of modafinil (1.0 μM) was observed on either the basal or the K^+-stimulated release of [^{3}H]GABA (Table 2).

3.2.2. *Effects of modafinil on the uptake of [^{3}H]GABA*

To exclude the possibility that the reduced cortical GABA outflow observed after the treatment with

Table 5
In vitro effects of modafinil on glutamic acid decarboxylase activity in extracts from the rat brain

Concentration of modafinil (μM)	GAD activity (nmol/mg protein per h)	
	+ pyridoxal phosphate	− pyridoxal phosphate
–	226 ± 20	192 ± 6.4
1	228 ± 8.4	206 ± 17
10	224 ± 20	195 ± 15
100	230 ± 11	207 ± 10
1000	252 ± 8.3	221 ± 3.1

The L-glutamate (75.6 μCi/mmol) concentration was 3.0 μM. Means ± S.E.M. are shown. See Materials and methods for details.

modafinil depended on changes in GABA uptake, the high-and low-affinity [^{3}H]GABA uptake was studied in slices of the rat frontoparietal cortex. As shown in Table 3 neither the total high affinity uptake of [^{3}H]GABA nor the glial [^{3}H]GABA uptake (measured in the presence of the neuronal [^{3}H]GABA uptake blocker L-2,4-diaminobutyric acid) was affected by modafinil (1–100 μM). Identical results were obtained when the effects of modafinil were studied on the low-affinity [^{3}H]GABA uptake (Table 4).

3.2.3. Effects of modafinil on glutamic acid decarboxylase activity

To further analyze the effects of modafinil on the GABAergic system, glutamic acid decarboxylase enzyme activity was measured in vitro in the presence of a large range of modafinil concentrations. As shown in Table 5 modafinil (1–1000 μM) failed to modify glutamic acid decarboxylase activity both in the presence and in the absence of pyridoxal phosphate. In these experiments care was taken to keep the glutamate concentration low (3 mM) to avoid any disinhibitory effect of the substrate on a possible competitive inhibition induced by modafinil.

4. Discussion

Previous studies have shown that the acute or chronic administration of modafinil reduces the GABA outflow from the guinea pig cerebral cortex through the activation of 5-HT$_2$ receptors (Tanganelli et al., 1992). The present data indicate that such a reduction also occurs in the rat and seems to require the integrity of the entire brain circuitry, since modafinil was unable to affect either the basal or the K^+-evoked [^{3}H]GABA release in cortical and hippocampal slices. Furthermore, the present in vivo release experiments indicate that acute and especially chronic modafinil treatment in 5,7-DHT-pretreated animals does not reduce, but increases endogenous cortical GABA outflow. This facilitatory action was cancelled by pretreatment of the guinea pigs with the α_1-adrenoceptor antagonist, prazosin. In addition, in these lesioned animals chronic modafinil treatment consistently increased noradrenaline levels and, in line with its neuroprotective action (Fuxe et al., 1992), antagonized the reduction of 5-HT levels induced by the neurotoxin 5,7-DHT. Taken together these results suggest the hypothesis of a complex monoamine-mediated influence of modafinil on the GABAergic structures.

It is well established that (i) locus coeruleus stimulation as well as noradrenaline administration both in vivo and in vitro exert an excitatory action on cortical GABA neurons via α_1-adrenoceptors (Beani et al., 1988; Beani et al., 1986) and (ii) noradrenaline increases the activity of 5-HT neurons (Clement et al., 1992). On the other hand, 5-HT exerts an inhibitory effect on the release of GABA (Limberger et al., 1986). Thus, the GABAergic neuronal structures seem to be under the influence of noradrenaline (direct excitatory and 5-HT-mediated) and/or of 5-HT (direct inhibitory). It is reasonable to assume that, in the normal animal, modafinil, by acting on the noradrenergic system (Duteil et al., 1990) indirectly activates inhibitory 5-HT neuronal systems, leading to reduction of the cortical GABA outflow. The relevance of a cortical noradrenergic tone for a 5-HT-mediated inhibition is proved by the suppression of the modafinil effect following 6-hydroxydopamine treatment (Tanganelli et al., 1994). The present results demonstrate that the 5,7-DHT treatment associated with an increase in cortical noradrenaline levels reverses the effects of modafinil, suggesting that noradrenaline neurons are now able to activate through α_1-adrenoceptors the cortical GABA neurons. In conclusion, the inversion of modafinil action after 5,7-DHT may be due to its effect on the noradrenergic system (Duteil et al., 1990), which becomes the predominant mechanism by which modafinil controls the cortical GABA outflow.

The possibility that the reduction in the cortical GABA outflow observed in the freely moving guinea pig and rat after modafinil treatment could be produced as a consequence of a direct inhibitory action of modafinil on GABA neurons is unlikely, since in vitro modafinil was unable to modify the basal and the K^+-evoked [^{3}H]GABA release. Furthermore, we can exclude that modafinil might decrease the cortical GABA outflow by influencing GABA uptake and/or the glutamic acid decarboxylase activity in view of our in vitro results, which have shown that modafinil failed to affect the high neuronal and the low glial affinity GABA uptake as well as the enzyme activity.

In conclusion, our results suggest that the balance between noradrenaline and 5-HT transmission plays an important role in the physiological control of the activity of GABA neurons. The action of modafinil on cortical GABA release seems to be dependent on this monoaminergic balance. The resulting modulation of the GABAergic activity of the cerebral cortex may help explain the clinical profile of this psychoactive drug, especially its control of the sleep-wakefulness cycle.

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