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RESEARCH PAPER

The anorexic agents, sibutramine and fenfluramine, depress $GABA_B$-induced inhibitory postsynaptic potentials in rat mesencephalic dopaminergic cells

Ada Ledonne[1,3], Luca Sebastianelli[2,3], Mauro Federici[3], Giorgio Bernardi[2,3] and Nicola Biagio Mercuri[2,3]

[1]*Università della Calabria, Dipartimento Farmaco-biologico, Arcavacata di Rende, Cosenza Italy,* [2]*Clinica Neurologica Università degli Studi di Roma 'Tor Vergata' Viale Oxford, Roma, Italy, and* [3]*IRCCS Fondazione 'S. Lucia', Via del Fosso di Fiorano, Roma, Italy*

Background and purpose: Nutrition is the result of a complex interaction among environmental, homeostatic and reward-related processes. Accumulating evidence supports key roles for the dopaminergic neurons of the ventral midbrain in regulating feeding behaviour. For this reason, in the present study, we have investigated the electrophysiological effects of two centrally acting anorexic agents, fenfluramine and sibutramine, on these cells.
Experimental approach: Rat midbrain slices were used to make intracellular recordings from dopaminergic neurons of the substantia nigra and the ventral tegmental area. Gamma-aminobutyric acid (GABA)-mediated synaptic transmission was assessed from the inhibitory postsynaptic potentials (IPSPs) mediated by $GABA_A$ and $GABA_B$ receptors.
Key results: Fenfluramine and sibutramine reduced, concentration-dependently, the $GABA_B$ IPSPs, without affecting the $GABA_A$-mediated potentials. This effect is presynaptic, as postsynaptic membrane responses induced by application of a $GABA_B$ receptor agonist, baclofen, were not affected by the two drugs. Furthermore, the selective 5-hydroxytriptamine 1B ($5\text{-}HT_{1B}$) receptor antagonist, SB216641, blocked the reduction of $GABA_B$ IPSPs caused by fenfluramine and sibutramine, indicating that the receptor mediating this effect is $5\text{-}HT_{1B}$.
Conclusions and implications: Two anorexic agents, fenfluramine and sibutramine, induced the activation of $5\text{-}HT_{1B}$ receptors located on presynaptic GABAergic terminals, thus reducing the release of GABA. This action can alter the strength of synaptic afferents that modify the activity of dopaminergic neurons, inducing neuronal excitation. Our results reveal an additional mechanism of action for fenfluramine and sibutramine that might contribute to reducing food intake, by influencing the pleasurable and motor aspects of feeding behaviour.
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Keywords: anorexic agents; dopaminergic neuron; dopamine; GABA; IPSP; eating disorder; obesity

Abbreviations: IPSPs, inhibitory postsynaptic potentials; SNpc, substantia nigra pars compacta; VTA, ventral tegmental area

Introduction

Feeding behaviour is the result of a complex interaction between environmental, homeostatic and reward-related stimuli occurring in distinct brain areas (Zheng and Berthoud, 2007). It is generally thought that while the hypothalamic neurons regulate the homeostatic components of eating, dopaminergic neurons of the ventral midbrain play a key role in hedonic and willing components of food intake (Palmiter, 2007). Accordingly, the mesencephalic cells, responding to environmental and internal cues, contribute to appropriate decision-making processes (Nicola, 2007). Regarding the complex signalling system regulating food intake and energy expenditure, many key molecules (hormones and classical neurotransmitters) act not only at the hypothalamic neuronal level but also on midbrain dopaminergic neurons (Palmiter, 2007). For instance, leptin, a peptide secreted by adipocytes to signal the amount of fat storage in the body, reduces the firing rate of dopaminergic neurons in the ventral tegmental area (VTA) (Hommel *et al.*, 2006). Furthermore, it has been reported that insulin, the crucial hormone for glucose utilization, not only mediates satiety by affecting the activity of hypothalamic cells, but also regulates the firing discharge of

Correspondence: Nicola Biagio Mercuri, Clinica Neurologica Università degli Studi di Roma 'Tor Vergata' Viale Oxford, 81. 00133 Roma, Italy. E-mail: mercurin@uniroma2.it
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midbrain dopaminergic neurons (Palmiter, 2007). Moreover, ghrelin, the peptide secreted by the empty gut to induce a meal, and orexin, a hypothalamic-secreted peptide, influence the activity of dopaminergic neurons (Abizaid *et al.*, 2006; Borgland *et al.*, 2006; Jerlhag *et al.*, 2007). As all these evidences support a key role of the mesencephalic dopaminergic cells in controlling the drive for food and food-related gratification (Berridge, 2007), here we have investigated the electrophysiological effects of two anti-obesity compounds, fenfluramine and sibutramine (Stallone and Levitsky, 1994; Ryan *et al.*, 1995), on these cells. Of note, fenfluramine is no longer available for therapeutic uses because of its pulmonary and cardiac adverse effects and so sibutramine is currently the only centrally acting drug administered for long-term treatment of obesity (Hainer *et al.*, 2006; Padwal and Majumdar, 2007; Halford *et al.*, 2007). Both compounds are appetite suppressants drugs that affect the serotoninergic transmission increasing, by different mechanisms, the synaptic concentration of 5-hydroxytriptamine (5-HT). In particular, fenfluramine induces 5-HT release through a carrier-mediated mechanism (Garattini *et al.*, 1975; Berger *et al.*, 1992; Schuldiner *et al.*, 1993; Crespi *et al.*, 1997). However, a Ca^{2+}-dependent exocytotic mechanism has been also proposed (Cinquanta *et al.*, 1997). Sibutramine, on the other hand, acts as a 5-HT re-uptake inhibitor, increasing the extracellular concentration of 5-HT through the inhibition of the serotonin transporter (SERT) in the plasma membrane (Gundlah *et al.*, 1997; Heal *et al.*, 1998; nomenclature follows Alexander *et al.*, 2008). Some evidence indicates that sibutramine and its primary and secondary metabolites also act as inhibitors of the corresponding dopamine transporter (DAT) (Nakagawa *et al.*, 2001).

It has been reported that 5-HT and 5-hydroxytryptaminergic drugs depress the inhibitory postsynaptic potential (IPSP) mediated by the $GABA_B$ receptors in dopaminergic cells, acting on presynaptic $5HT_{1B}$ receptors (Johnson *et al.*, 1992; Sugita *et al.*, 1992). In this work, because of the importance of the dopaminergic neurons in the regulation of feeding behaviour, we have investigated how fenfluramine and sibutramine affect the activity of these cells in the substantia nigra pars compacta (SNpc) and VTA, and in particular their effects on $GABA_B$ IPSPs.

Methods

Slice preparation

All experiments were carried out in accordance with the international guidelines on the ethical use of animals from the European Communities Council Directive of 24 November 1986 (86/609/EEC). Wistar male rats (18–25 days old) were anesthetized by inhalation of 2-bromo-2-chloro-1,1,1-trifluoroethane and decapitated. The electrophysiological recordings were made from dopaminergic neurons of SNpc and VTA in acute slices of ventral midbrain obtained using standard procedures (Mercuri *et al.*, 1995). Briefly, the brain was rapidly removed from the skull and a tissue block containing the midbrain was mounted on an agar block and immersed in cold artificial cerebrospinal fluid (aCSF) at 8–10°C. The aCSF contained 126 mmol·L^{-1} NaCl, 2.5 mmol·L^{-1} KCl, 1.2 mmol·L^{-1} $MgCl_2$, 2.4 mmol·L^{-1} $CaCl_2$, 1.2 mmol·L^{-1} NaH_2PO_4, 24 mmol·L^{-1} $NaHCO_3$ and 10 mmol·L^{-1} glucose and was saturated with 95% O_2–5% CO_2 (pH 7.4 ± 0.02). Horizontal slices (250 μmol·L^{-1}) of the ventral midbrain, containing the substantia nigra and the VTA, were cut using a vibratome (Leica VT1000S, Leica Microsystems, Wetzlar, Germany). Slices were maintained in aCSF at 33.0 ± 0.5°C for 45 min before being transferred in the recording chamber.

Electrophysiology

Intracellular recordings from midbrain SNpc and VTA dopaminergic neurons were performed at 33.0 ± 0.5°C in a recording chamber submerged with aCSF flowing at a rate of 2.5–3 mL·min^{-1} and continuously oxygenated, on the stage of an upright (inverted) microscope (Axioscope FS, Zeiss, Gottingen, Germany), equipped for infrared video microscopy (Hamamatsu, Tokyo, Japan) in order to allow a direct visualization of the recorded cells. Neurons, selected for their morphology, were identified as dopaminergic by their electrophysiological properties such as the presence of a regular spontaneous firing activity (0.5–4 Hz), a large inward current (Ih) in response to hyperpolarizing voltages and a membrane hyperpolarization due to dopamine (10–30 μmol·L^{-1}) application (Grace and Onn, 1989; Mercuri *et al.*, 1995). The recording electrodes were filled with 2 mol·L^{-1} KCl and had a tip resistance of 30–80 MΩ.

$GABA_B$ synaptic potentials were evoked using a bipolar tungsten stimulating electrode with a tip separation of 300–700 μm (Johnson and North, 1992; Johnson *et al.*, 1992; Sugita *et al.*, 1992). To prevent spontaneous spikes, the membrane potential was adjusted between –65 and –70 mV by hyperpolarizing current injection. A train of four to eight stimuli of 70 μs at 8–20 V was delivered at 70 Hz every 25 s. Stimulating electrodes were placed within 500–700 μm rostral or caudal to the recording electrode. The amplitude of the evoked synaptic potential was measured from traces that represent the average of four recorded responses.

$GABA_B$ mediated IPSPs were isolated using a pharmacological cocktail containing bicuculline methiodide (30 μmol·L^{-1}), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μmol·L^{-1}), 2-amino-5-phosphopentanoic acid (APV, 50 μmol·L^{-1}) and sulpiride (1 μmol·L^{-1}) to block $GABA_A$, amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), *N*-methyl-D-aspartate (NMDA) and dopamine D_2 receptors respectively. In some experiments, the $GABA_B$ receptor antagonist, CGP-55845 (1 μmol·L^{-1}), was perfused to block $GABA_B$ IPSPs. The $GABA_A$ IPSPs were evoked by a single electrical stimulus with the same cocktail used to evoke the $GABA_B$ IPSPs but omitting bicuculline. In some experiments, the same protocol of stimulation necessary to evoke the $GABA_B$ IPSPs was used, in the presence of CGP 55845 (1 μmol·L^{-1}), to generate a giant $GABA_A$ potential.

For the experiments performed to directly activate postsynaptic $GABA_B$ receptors, baclofen (30 μmol·L^{-1}) was applied in the bath for only 5–7 s in order to induce a hyperpolarization similar in amplitude to the evoked $GABA_B$ IPSP.

Data analysis

Numerical data were expressed as mean ± standard error of the mean (SEM). Student's *t*-test for paired observations was

used to compare the data. A $P < 0.05$ was considered to be significant. The percentage change produced by a drug was calculated from mean amplitude of four responses before and after the equilibrium had been reached. To estimate the IC_{50} and maximal response, concentration-response curves were fitted using the logistic equation $y = \frac{A_1 - A_2}{1 + (x/x_0)^P} + A_2$ where x_0 is centre, A_1 is initial y value, A_2 is final y value and p is power.

Drugs

All drugs were prepared in stock solutions and applied in the bath at known concentrations via a three-way tap system. A complete exchange of the solution in the recording chamber occurred in about 1 min. Dopamine hydrochloride, CGP 55845, AP-5, bicuculline methiodide, (+/–)fenfluramine and sulpiride were purchased from Sigma, (Milan, Italy). Sibutramine hydrochloride, CNQX, baclofen and SB 216641 were obtained from Tocris Cookson Inc. (Bristol, UK). 5-HT (serotonin creatinine sulphate) was provided by Merck (Darmstadt, Germany).

Results

Fenfluramine and sibutramine reduce, in a concentration-dependent manner, the $GABA_B$ but not the $GABA_A$ IPSPs

Intracellular recordings with sharp microelectrodes were made from presumed dopaminergic neurons in the rat SNpc ($n = 8$) and VTA area ($n = 20$) maintained *in vitro* in brain slices whose electrophysiological and pharmacological characteristics have been already described (Grace and Onn, 1989; Lacey *et al.*, 1989; Johnson and North, 1992; Mercuri *et al.*, 1995).

Fenfluramine (as racemic mixture) and sibutramine (0.1–100 μmol·L^{-1}) did not affect the firing activity and the membrane potential of the dopaminergic neurons (data not shown). Most of the firing data were obtained with maximal doses of both compounds (100 μmol·L^{-1}) in eight cells for each compound. The apparent input resistance of the neurons, measured by 10–15 mV hyperpolarizing pulses, was not changed by the application of the two anorexic agents (120 ± 18 MΩ in control condition; 123 ± 14 MΩ in fenfluramine; 121 ± 20 MΩ in sibutramine, $P > 0.05$) (not shown).

We then examined the effects of the two anorexic drugs on the slow inhibitory synaptic transmission mediated by $GABA_B$ receptors. $GABA_B$ IPSPs (8–20 mV) were evoked in neurons by a local short train of stimuli. To confirm the involvement of $GABA_B$ receptors in the generation of the slow IPSPs, we have abolished these potentials by using CGP 55845 (1 μmol·L^{-1}), a $GABA_B$ antagonist (Figure 1A,B). The electrophysiological and pharmacological characteristics of these inhibitory potentials in the VTA or in the SNpc were similar, so that the data were pooled.

Fenfluramine, perfused at a concentration of 0.1–1000 μmol·L^{-1}, reduced the amplitude of the $GABA_B$ IPSPs (Figure 1A). This effect had a slow onset, peaked in 6 to 18 min and did not entirely recover after more than 1 h of washing ($n = 16$). The reduction of the IPSP was

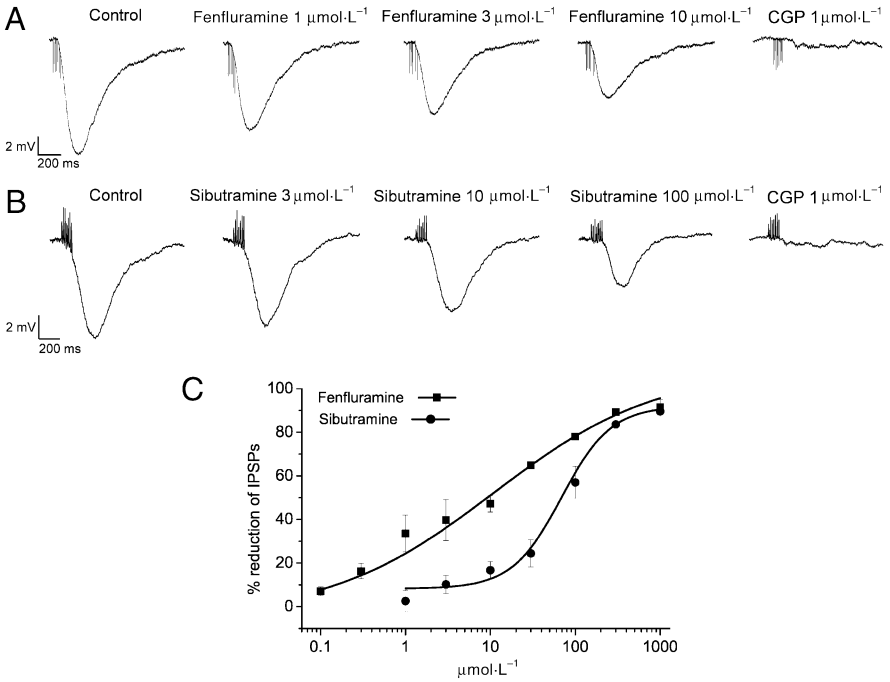

Figure 1 Fenfluramine and sibutramine induce a concentration-dependent reduction of the $GABA_B$-mediated inhibitory postsynaptic potentials (IPSPs). (A) Representative recordings showing the effect of fenfluramine in reducing the $GABA_B$-mediated IPSP. Such effects were evident at concentrations below 1 μmol·L^{-1}. The amplitude of synaptic potentials was halved by fenfluramine at concentration around 10 μmol·L^{-1} and was completely blocked by CGP (1 μmol·L^{-1}), a selective $GABA_B$ receptor antagonist. (B) Sibutramine also induced a reduction of the $GABA_B$-mediated IPSP. Compared with that of fenfluramine, this effect is less evident at low concentrations. Concentrations of sibutramine less than 100 μmol·L^{-1} are able to halve the $GABA_B$ IPSP. (C) From the concentration-response curves shown, values of IC_{50} of 11 ± 3 μmol·L^{-1} for fenfluramine and 69 ± 13 μmol·L^{-1} for sibutramine have been calculated ($n = 3$–8 cells per concentration for fenfluramine and 3–5 for sibutramine). CGP55845.

concentration-dependent having an IC_{50} of 11 ± 3 μmol·L^{-1} (Figure 1A,C). The maximal inhibitory effect during the perfusion of fenfluramine (1000 μmol·L^{-1}) was a reduction by 92 ± 3% of the amplitude of the $GABA_B$ IPSP ($n = 3$). Sibutramine (1–1000 μmol·L^{-1}) also caused a concentration-dependent depression of $GABA_B$ IPSPs (Figure 1B,C) that did not wash out. The effect of sibutramine, having an IC_{50} of 69 ± 13 μmol·L^{-1} (Figure 1C), was weaker than that of fenfluramine; however, the highest concentration tested (1000 μmol·L^{-1}) almost abolished the IPSPs. Therefore, these two compounds, at maximal concentrations, reduced the amplitude of the slow IPSPs to a similar extent (Figure 1C).

As reported for 5-HT and for the prototypical 5-HT uptake (SERT) blocker, cocaine (Johnson *et al.*, 1992; Sugita *et al.*, 1992; Cameron and Williams, 1994), fenfluramine and sibutramine were able to reduce only the slow component ($GABA_B$ receptor-mediated) of the inhibitory synaptic transmission. In fact, the fast inhibitory component, mediated by the activation of $GABA_A$ receptors, was not affected (Figure 2). Thus, the amplitude of the $GABA_A$ IPSPs in control condition (normalized, absolute value 9.7 ± 0.8 mV, $n = 9$) was not significantly modified by fenfluramine ($n = 5$, $P = 0.58$) (Figure 2A,C) at the concentration (100 μmol·L^{-1}) which almost abolished the $GABA_B$ IPSPs (Figure 2B,C). Similarly, sibutramine (100 μmol·L^{-1}) did not affect the $GABA_A$ IPSPs ($n = 5$, $P = 0.79$) (Figure 2D,F), but clearly depressed the $GABA_B$-mediated component of the inhibitory transmission (Figure 2E,F).

To exclude the possibility that this difference might depend on the different protocol used to evoke $GABA_A$ and $GABA_B$ IPSP, additional experiments were performed evoking longer $GABA_A$ IPSPs with the same train protocol used for $GABA_B$ potentials. These experiments confirmed a selective effect of fenfluramine on the $GABA_B$ IPSP, because, when it was tested (100 μmol·L^{-1}) on the longer $GABA_A$ potential (7.5 ± 0.6 mV, 325 ± 39 ms, $n = 3$), it did not change the

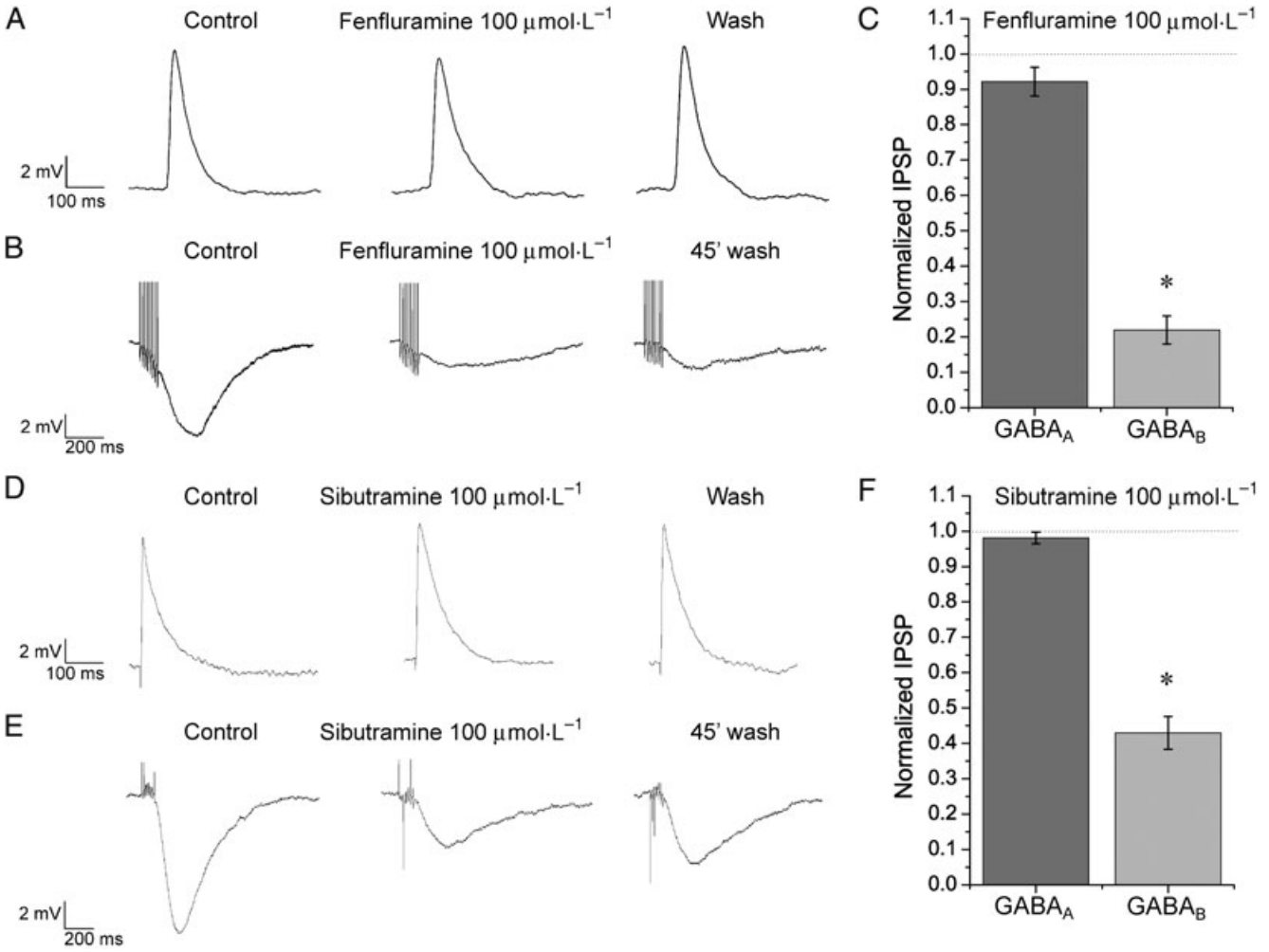

Figure 2 Fenfluramine and sibutramine did not reduce $GABA_A$ IPSP. Fenfluramine (100 μmol·L^{-1}) did not significantly affect the $GABA_A$ IPSP (A), while it strongly reduced the $GABA_B$ IPSP (B). In (C), the mean values of the effects of fenfluramine on both $GABA_A$ and $GABA_B$ IPSPs are shown, expressed as normalized percent reduction. Similarly, the application of sibutramine (100 μmol·L^{-1}) did not change the $GABA_A$ IPSP (D), whereas it reduced the $GABA_B$ IPSP (E). Mean values confirming the selectivity of both drugs for $GABA_B$ over $GABA_A$ are shown in (F). *$P < 0.05$, significantly different from effects on $GABA_A$ IPSP. GABA, gamma-aminobutyric acid; IPSP, inhibitory postsynaptic potential.

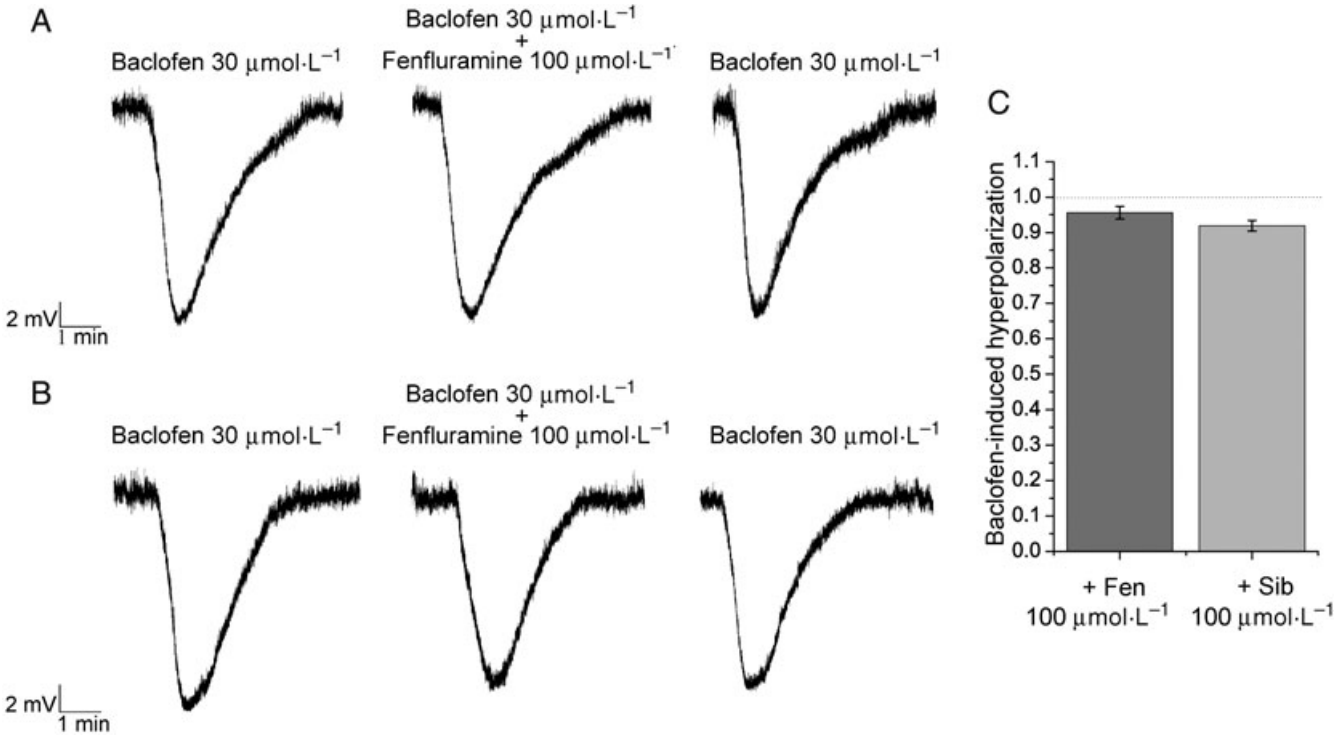

Figure 3 The blocking effect of fenfluramine and sibutramine on $GABA_B$ IPSPs was exerted presynaptically. Exogenous application of the $GABA_B$ receptor agonist, baclofen (30 μmol·L^{-1}), induced a hyperpolarization that was unaffected by fenfluramine (100 μmol·L^{-1}) (A) or sibutramine (100 μmol·L^{-1}) (B). This suggests that both drugs act presynaptically to reduce the release of GABA. Mean values of the effects of both drugs on the normalized baclofen-induced hyperpolarization are shown in (C). GABA, gamma-aminobutyric acid; IPSP, inhibitory postsynaptic potential.

amplitude of this synaptic event ($n = 3$, $P = 0.74$) (data not shown).

Fenfluramine and sibutramine did not modify the postsynaptic responses to baclofen

To assess whether the effects of fenfluramine and sibutramine on the $GABA_B$-mediated transmission were pre- or postsynaptic, a direct activation of postsynaptic $GABA_B$ receptors was induced by limited application of the $GABA_B$ agonist baclofen. We observed that the hyperpolarization caused by short exposure (5–7 s only) to baclofen (30 μmol·L^{-1}) (9.1 ± 0.4 mV, $n = 9$) was not significantly modified by the superfusion of fenfluramine (100 μmol·L^{-1}, 8.7 ± 0.4 mV, $n = 5$, $P = 0.38$) (Figure 3A). Also sibutramine (100 μmol·L^{-1}) did not change the membrane effects due to the baclofen-induced postsynaptic activation of $GABA_B$ receptors ($n = 5$, $P = 0.12$) (Figure 3B).

The effects of fenfluramine and sibutramine were due to activation of 5-HT_{1B} receptors

It is widely reported that both fenfluramine and sibutramine increase the synaptic concentration of 5-HT. Fenfluramine induces carrier-mediated 5-HT release while sibutramine seems to act as a 5-HT uptake inhibitor (Heal *et al.*, 1998). It has been previously demonstrated that 5-HT reduces $GABA_B$ IPSPs in the dopaminergic neurons of SNpc and VTA (Sugita *et al.*, 1992) through the activation of presynaptic 5-HT_{1B} receptors located on GABAergic terminals (Johnson *et al.*, 1992). In order to verify the hypothesis that the reduction of the $GABA_B$ IPSPs caused by fenfluramine or sibutramine were due to the activation of 5-HT_{1B} receptors, we selectively blocked these, using the antagonist SB 216641.

In our experiments, SB 216641 (30 μmol·L^{-1}) applied in the bath for 15 min, antagonized the effects of 5-HT, by preventing the reduction of $GABA_B$ IPSPs observed in control conditions (Figure 4A; mean values in Figure 4D; $n = 5$, $P = 0.0017$). During superfusion with this antagonist, inhibition of the $GABA_B$ IPSPs produced by 5-HT (10 μmol·L^{-1}) was only about 10% of control (Figure 4D). Furthermore, this concentration of SB216641 was as effective in blocking the effects of fenfluramine (100 μmol·L^{-1}) and sibutramine (100 μmol·L^{-1}) on the $GABA_B$ IPSPs (Figure 4B,C; mean values in Figure 4D; $n = 3$ for each, $P < 0.05$).

Fenfluramine and sibutramine did not modify the postsynaptic responses to dopamine

Neither fenfluramine (100 μmol·L^{-1}) nor sibutramine (100 μmol·L^{-1}) ($n = 3$ for each drug) affected the membrane hyperpolarizations caused by the application of exogenous dopamine (30 μmol·L^{-1}) on the dopaminergic neurons. The control hyperpolarization to dopamine was 8 ± 2.5 mV ($n = 6$); in the presence of fenfluramine, hyperpolarization was 7.8 ± 2.4 mV ($n = 3$, $P > 0.05$) and in the presence of sibutramine, it was 8.2 ± 1.5 mV ($n = 3$ $P > 0.05$).

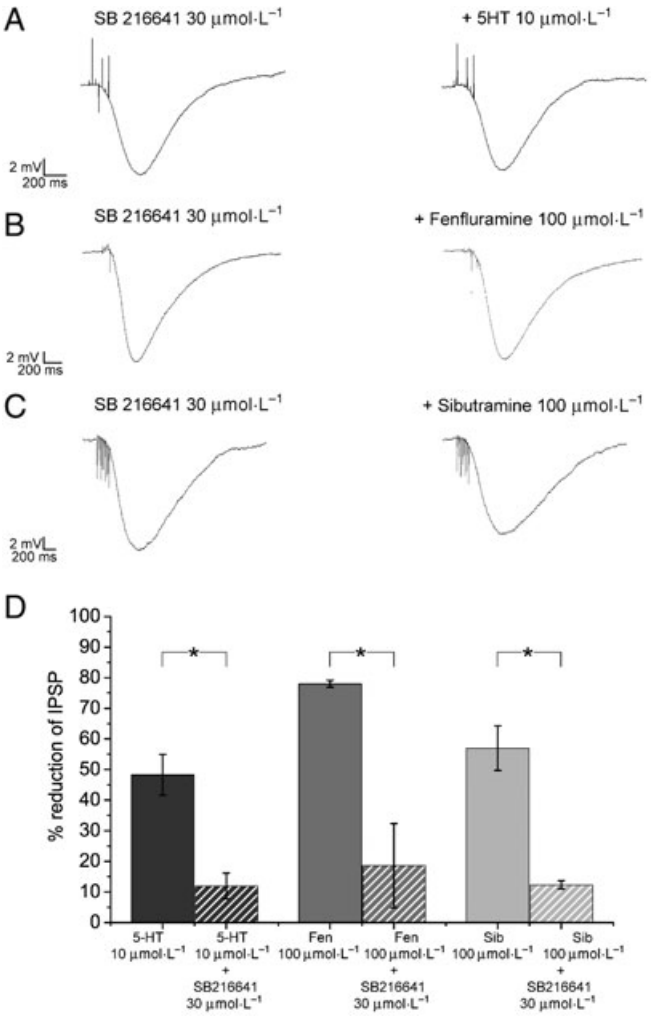

Figure 4 The effect of fenfluramine and sibutramine was mediated by the activation of 5-HT_{1B} receptors. Representative traces showing the effects of 5-HT, fenfluramine and sibutramine on $GABA_B$ IPSP in presence of the selective 5-HT_{1B} receptor antagonist SB216641. (A) The superfusion of SB 216641 (30 μmol·L^{-1}) almost completely blocked the reduction of $GABA_B$ IPSPs induced by 5-HT (10 μmol·L^{-1}). Under the same conditions, the inhibitory effects of fenfluramine (100 μmol·L^{-1}) (B) and sibutramine (100 μmol·L^{-1}) (C) are abolished. Mean values of the effects of SB216641 are shown in (D) as percent reduction of the control IPSP. *Significant effect of SB216641, $P < 0.05$. 5-HT, 5-hydroxytriptamine; GABA, gamma-aminobutyric acid; IPSP, inhibitory postsynaptic potential.

Discussion

Using *in vitro* intracellular electrophysiological recordings of midbrain dopaminergic cells, here we have found that two appetite-suppressant drugs, fenfluramine and sibutramine, exert a potent and long-lasting depressant effect on the $GABA_B$ IPSPs. Therefore, the consequent attenuation of GABA transmission facilitates neuronal activation and, subsequently, the release of dopamine in crucial areas. Thus, the increase in firing activity associated with increased levels of dopamine in the brain might contribute to produce 'chemical' satisfaction and reward that very likely reduced the drive for food (i.e. the amount of time and effort that an individual invests to obtain food).

It is known that diverse dopaminergic pathways exist in the CNS, regulating goal directed tasks and the addictive properties of many substances and foods. Interestingly, fenfluramine and sibutramine induced a depression of the $GABA_B$ IPSP in neurons from both the dopaminergic midbrain areas, VTA and SNpc, hence activating the meso-accumbens and nigrostriatal pathways respectively. This is in agreement with the hypothesis (Palmiter, 2007) that dopamine signalling is important for feeding behaviour both in the ventral and in the dorsal striatum. The extracellular concentration of dopamine (measured with *in vivo* microdialysis) is increased following systemic administration of sibutramine or fenfluramine, both in the striatum (De Deurwaerdère *et al*., 1995; Balcioglu and Wurtman, 1998; 2000) and in the nucleus accumbens (Rowley *et al*., 2000) of freely moving rats.

In spite of the fact that an effect on firing could not be detected under our experimental conditions, very likely because the *in vitro* slice preparation alters the synaptic circuitry, it is conceivable that the effects on $GABA_B$ potentials caused by the two anorexic agents could affect firing rate *in vivo*. It has been demonstrated that the increased dopamine release in the projecting areas is dependent on activation of firing in the dopaminergic neurons (De Deurwaerdère *et al*., 1995; Ukai *et al*., 2004). An increase in the somatodendritic release of dopamine in the SNpc following fenfluramine local injection has also been described (Cobb and Abercrombie, 2003). Such an increase in dopamine concentration might be attributable to inhibition of the DAT by these anorexic agents (Nakagawa *et al*., 2001). However, our observation that the membrane hyperpolarization caused by the application of exogenous dopamine was unaffected by fenfluramine would not support a blockade of DAT, in our model.

Our results are compatible with presynaptic effects of fenfluramine and sibutramine, mediated by 5-HT. Indeed, these compounds did not change the passive properties of dopaminergic cells (membrane resistance and membrane potential) or the postsynaptic responses to baclofen. In addition, we prevented the drug-induced depression of the $GABA_B$ IPSP, by antagonism of $5HT_{1B}$ receptors which could be located on presynaptic GABAergic terminals (Bruinvels *et al*., 1993). The control by compounds acting on 5HT receptors of the $GABA_B$ receptor-mediated inputs might also regulate the somatodendritic release of dopamine from dopaminergic neurons (Klitenick *et al*., 1992).

Thus, fenfluramine and sibutramine, increasing the release or inhibiting the re-uptake of 5-HT respectively, (Gundlah *et al*., 1997; Heal *et al*., 1998; John and Jones, 2007), exert a dis-inhibitory effect, through $5HT_{1B}$ presynaptic receptors. This specificity of action is also supported by the observation that only the $GABA_B$ and not the $GABA_A$ IPSP was affected. This is consistent with previous studies (Johnson *et al*., 1992; Sugita *et al*., 1992; Cameron and Williams, 1994) describing the effects of 5-HT and 5-hydroxytryptaminergic drugs on dopaminergic neurons and supports a segregation of synaptic inputs to $GABA_B$ and $GABA_A$ receptors on dopaminergic neurons (Sugita *et al*., 1992).

Fenfluramine had an EC50 6.3 times lower than that of sibutramine (fenfluramine EC50 = 10.9 ± 3.4 μmol·L^{-1}; sibutramine EC50 = 68.9 ± 13.2 μmol·L^{-1}) in our experiments. Of note, both EC50 were in the micromolar range,

comparable to the concentrations of cocaine effective on SERT (Cameron and Williams, 1994; Lacey *et al*., 1990). With regard to the effects of sibutramine, there are experimental data showing that the blockade of SERT is mainly caused by its metabolites (Heal *et al*., 1998). However, the present study, showing that applications of sibutramine *in vitro* rapidly depressed the $GABA_B$ IPSP, suggested a direct action of this compound, independent of active metabolites.

The importance of $5HT_{1B}$ receptors in tuning the activity of the meso-limbic pathway, increasing the level of dopamine in the nucleus accumbens and thus regulating reward-seeking behaviour, has been already suggested (O'Dell and Parsons, 2004). An increased level of dopamine in the ventral mesencephalon and terminal areas, by altering normal dopaminergic transmission, might contribute to the inhibitory effects of the two drugs on feeding (Palmiter, 2007). An exclusive role of 5-HT in reward is also supported by recent data showing that the blockade of SERT activates dopaminergic neurons in transgenic mice deficient in dopamine (Hnasko *et al*., 2007).

In line with this, the present paper has demonstrated presynaptic depressant effects of fenfluramine and sibutramine on the synaptic release of GABA, mediated by $5\text{-}HT_{1B}$ receptors, in midbrain dopaminergic cells, via modification of 5-HT uptake/release processes. We believe that the resultant variation of neuronal activity could participate in the internal signals that control food-reward and, eventually, hunger and satiety.

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Conflict of interest

None.

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