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4-methyl benzylamine stimulates food consumption and counteracts the hypophagic effects of amphetamine acting on brain *Shaker-like* Kv1.1 channels

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1 4-methyl benzylamine (4-MBZ; 28 μg, 231 nmol) elicits a hyperphagic response in starved mice in contrast to the hypophagia induced by the parent compound benzylamine (BZ; 33 μg, 231 nmol) or by amphetamine (AMPH, 2 μg).

2 In mice starved for only 4 h, and therefore with little stimulation to eat, the maximal increase in food consumption induced by intracerebroventricular (i.c.v.)-injected 4-MBZ was 190% over that of the controls (ED_{50} $8.3 \pm 2.7\,\mu g\,mouse^{-1}$; $68 \pm 22\,nmol\,mouse^{-1}$), whereas after i.p. administration, these values were 160% and approximately $129\,mg\,kg^{-1}$, respectively.

3 The hyperphagic effect of 4-MBZ was reduced by more than 60% in mice pretreated with antisense oligodeoxyribonucleotide ($aODN_1$) previously found to selectively inhibit (over 50%) the expression of *Shaker-like* Kv1.1 channels.

4 In mice highly stimulated to eat after 12-h fasting, 4-MBZ (28 μg) significantly reduced (to about 70%) the hypophagic response by AMPH (2 μg) or BZ (33 μg). Conversely, these two compounds reduced (respectively, by 69 and 44%) the hyperphagic response of 4-MBZ in 4-h fasting mice.

5 4-MBZ (28 μg) also reduced the hypermotility and the stimulation of inspection activity elicited by AMPH in mice and the release of DA stimulated by AMPH (2 μg) from the nucleus accumbens of rats.

6 We hypothesize that 4-MBZ elicits hyperphagic effects probably by opening *Shaker-like* Kv1.1 subtypes in the brain, whereas AMPH and BZ are hypophagic by blocking these channels.
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Abbreviations: AMPH, amphetamine; $aODN_1$, oligodeoxyribonucleotide anti-Kv1.1; BZ, benzylamine; 4-MBZ, 4-methyl benzylamine

Introduction

Amphetamine (AMPH), an indirect sympathomimetic compound, elicits its neurobehavioural effects including anorexia, stimulation of physical performance, insomnia, euphoria, etc. by releasing DA, 5-HT and other neurotransmitters into the brain. The lateral hypothalamus, nucleus accumbens and midbrain raphe nucleus are the regions in which food intake, motor activity, analgesia and arousal are known to be influenced (Parada *et al*., 1988; Inui, 2000; Hoffman, 2001). AMPH-stimulated exocytosis involves a reverse transport from synaptic vesicles to cytosol, the elevation of intragranular pH and disruption of the association of catecholamines with Ca^{2+}, ATP and vesicular proteins (Sulzer & Rayport, 1990; Sulzer *et al*., 1995; Sonders *et al*., 1997; Mundorf *et al*., 1999). Recent investigations, however, in mice pretreated with antisense oligonucleotides have shown that the stimulant properties of AMPH also require the full expression of *Shaker-like* Kv1.1 potassium channels in the brain (Ghelardini *et al*., 2003).

Benzylamine (BZ), a compound endowed with a phenylmethylamine skeleton shorter than the phenylethylamine structure of AMPH, also elicits central hypophagia in starved mice still requiring the full expression of Kv1.1 potassium channel subtypes in the brain (Ghelardini *et al*., 1997; Banchelli *et al*., 2000; 2001; Pirisino *et al*., 2001). In recent studies aimed at comparing the potential hypophagic effects of some BZ derivatives, we observed that, at condition of maximal food intake stimulation (12 h starved mice), 4-methyl benzylamine (4-MBZ) administration elevated, albeit not significantly, mice food intake, thus showing an opposite effect to that of the parent compound BZ or of the AMPH (Raimondi *et al*., 2003).

These observations suggested that the phenylmethylamine skeleton may represent a chemical structure endowed with the unique ability to differently modulate the brain *Shaker-like* Kv1.1 channel subtypes because of the different substituents present in the molecule. In particular, it was reasonable to find out whether 4-MBZ, due to its putative properties on potassium channels, was also able to counteract other central stimulant effects of AMPH.

In the present work, we investigated whether (i) 4-MBZ also elicited hyperphagic effects in mice not maximally stimulated to eat (4 h of fasting) in order to better evaluate the increase in

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food consumption; (ii) the hyperphagic effect of 4-MBZ was also dependent on the complete expression in the brain of Kv1.1 channel subtypes; (iii) 4-MBZ and BZ or AMPH reciprocally counteracted their own central effects on food consumption and whether 4-MBZ counteracted the motor stimulant effects of AMPH; (iv) BZ, like AMPH, was characterized, in microdialysis experiments, by central dopaminergic activity and whether 4-MBZ also inhibited the DA release stimulated by AMPH from the nucleus accumbens of freely moving rats.

Methods

Animals

Male Swiss albino mice (24–26 g) and albino rats (150–200 g) from Morini (San Polo d'Enza, Italy) were used. In all, 15 mice or five rats were housed per cage. The cages were placed in the experimental room 24 h before the test for acclimatization purposes. The animals were fed a standard laboratory diet and tap water *ad libitum*; they were kept at $23 \pm 1°C$, with a 12 h light/dark cycle, and lights on at 0700 hours. All experiments were carried out in accordance with the European Community Council's Directive of 24 November 1986 (86/609/EEC) relative to experimental animal care. All efforts were made to minimize animal suffering and to reduce the number of animals used (in any case, unless otherwise indicated, at least 10 animals per group were used in each behavioural protocol).

Evaluation of food consumption

The mice did not have access to food for 4 or 12 h but water was available *ad libitum*. A weighed amount of food (standard laboratory pellets) was given, and the amount consumed (evaluated as the difference between the original amount and the food left in the cage, including spillage) was measured 15, 30, 45 and 60 min after intracerebroventricular (i.c.v.) or i.p. administration of saline or drug solutions, with an accuracy of 0.1 g. Considering that, as it was previously described (Ghelardini *et al*., 1997), after 60 min of food readmission, the consumption reached a plateau, a cutoff time of 60 min was used, and the total amount of food consumed was expressed in $mg\,mouse^{-1}\,h^{-1}$. In single-dose experiments on food consumption, BZ and AMPH were given i.c.v. at the equiactive doses of 33 μg (231 nmol) and 2 μg, respectively, while 4-MBZ (28 μg; 231 nmol) was given at the same molar concentration of BZ. In dose–response experiments i.p.-injected 4-MBZ ranged from 10 to 600 $mg\,kg^{-1}$ and from 1.2 to 54.8 μg (10–452 nmol) when given i.c.v.

Hole-board test

The hole-board test consisted of a 40 cm square plane with 16 flush-mounted cylindrical holes (3 cm diameter) distributed 4 by 4 in an equidistant, grid-like manner. Mice were placed one by one on the centre of the board and allowed to move about freely for a period of 10 min each. Two photoelectric cells, recording a beam of light crossing the plane from mid-point to mid-point of opposite sides (thus dividing the plane into four equal quadrants), automatically signalled the movement of the animal (counts $5\,min^{-1}$) on the surface of the plane (locomotor activity). Miniature photoelectric cells in each of the 16 holes recorded (counts $5\,min^{-1}$) exploration of the holes (exploratory activity) by the mice. At least 12 mice per group were tested.

Drug administration by i.c.v. route

The i.c.v. administration was performed under ether anaesthesia with isotonic saline used as solvent, according to the method described by Haley & Mccormick (1957). During anaesthesia, the mice were grasped firmly by the loose skin behind the head. A hypodermic needle (0.4 mm external diameter) attached to a 10 μl syringe was inserted perpendicularly through the skull and no more than 2 mm into the brain of the mouse, where a 5 μl solution was then administered. The injection site was 1 mm to the right or left of the midpoint on a line drawn through to the anterior base of the ears. Injections were performed randomly into the right or left ventricle. To ascertain that solutions were administered exactly into the cerebral ventricle, some mice were injected with 5 μl of diluted 1:10 India ink and their brains were examined macroscopically after sectioning. The accuracy of the injection technique was evaluated, with 95% of injections being correct.

Antisense oligonucleotides

24 mer phosphodiester oligonucleotides (ODNs) were capped by a terminal phosphorothioate double substitution and purified by high-performance liquid chromatography (HPLC; Genosys, The Woodlands, TX, U.S.A.). The $aODN_1$ (5′-CGA CAT CAC CGT CAT GAT GAA AGG-3′) was designed by targeting the 5′ portion of the murine Kv1.1 mRNA, residues 575–598 of the published cDNA sequence (Chandy *et al*., 1990).

To evaluate the specific antisense effects of the oligodeoxynucleotides, a fully degenerated phosphorodiester phosphorothioate-capped oligonucleotide ($dODN_1$) was used as a negative control. The fully degenerated 24 mer is a collection of about 3×1014 different molecular species (5′-NNN NNN NNN NNN NNN NNN NNN NNN-3′; where N = G, C, A or T). Therefore, for the nanomolar–micromolar range concentrations used in the antisense experiments, the dODN was present at the site of action in a sub-attomolar concentration, which is totally insufficient for any antisense effect. For more details on the $aODN_1$ and $dODN_1$ synthesis and the RT–PCR analysis of mKv1.1 mRNA in the mouse brain tissues, the reader may refer to our previous papers (Ghelardini *et al*., 1997; 2003; Meiri *et al*., 1997; Galeotti *et al*., 1997a, b).

Administration of antisense oligonucleotides

Phosphorothioate-capped phosphorodiester oligonucleotides associated with an artificial cationic lipid (DOTAP = *N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium methyl sulphate), which proved to be without effect on the behavioural parameters under investigation, were used (Ghelardini *et al*., 2003). Mice were randomly assigned to an antisense oligonucleotide ($aODN_1$), degenerated oligonucleotide ($dODN_1$), vector (DOTAP), saline or a naïve group. A suitable amount of oligonucleotide was preincubated at 37°C for 30 min with 13 μM DOTAP. Each group received a single i.c.v. injection (5 μl; 3 nmol of ODN) on days 1, 4 and 7. All behavioural tests

were performed 48 h after the last i.c.v. injection of $aODN_1$ or $dODN_1$, when the maximal inhibition in the expression of mKv1.1 mRNA in the mouse brain tissue was detectable using RT–PCR analysis (Ghelardini *et al*., 1997; Galeotti *et al*., 1997a, b). As it had been previously assessed (Galeotti *et al*., 1997a, b) that $dODN_1$ did not induce any evident modification in the basal behavioural function of mice, as compared to saline or vector-injected animals, $dODN_1$-treated animals were used as controls in all experiments.

Microdialysis procedures

Rats received sodium pentobarbital ($60\,mg\,kg^{-1}$, i.p.) as surgical anaesthesia. Each rat was then placed in a stereotaxic apparatus for craniotomy. A plastic intracerebral guide cannula (CMA 12, CMA/Microdialysis, Acton, MA, U.S.A.) was implanted above the nucleus accumbens (ML + 1.5 mm and AP + 1.6 mm from bregma, DV-8.2 mm from skull). Animals were housed singly and allowed 7–10 days to recover and microdialysis was carried out with a probe of $2\,mm \times 0.5\,mm$ (CMA/12, CMA/Microdialysis) placed inside the guide cannula by following the method described by Baumann *et al*. (2000). Each rat was placed in a bowl, where free movements were allowed. Ringers' solution, containing 147.0 mM NaCl, 4.0 mM KCl and 1.8 mM $CaCl_2$, was pumped through the probe at $0.5\,\mu l\,min^{-1}$. Beginning 2–3 h after the insertion of the probe, dialysate samples were collected at 20-min intervals and immediately assayed for DA by HPLC using electrochemical detection. After DA stabilization in dialysate, three baseline samples were collected and DA concentrations were expressed as a percentage of the baseline. AMPH, BZ or 4-MBZ were administered in rats *via* reverse-dialysis, in a single dose of 2, 33 or 28 μg, respectively, 60 min after probe insertion; when antagonism experiments were performed, AMPH 2 μg and 4-MBZ 28 μg were coadministered with the same procedure. Samples were collected for 280 min after the start of the experiments. At the end of the experiments, the location of the probes in the brain was evaluated by means of histological analysis. The data included in this study refer to rats bearing probes correctly placed within the nucleus accumbens.

Analysis of DA in dialysate samples

Aliquots of the dialysate (5 μl) were injected directly into a microbore HPLC column coupled to an amperometric detector. A glass carbon working electrode was set at a potential of + 700 mV relative to an Ag/AgCl reference. A mobile phase consisting of 14.2 g monochloroacetic acid, 6.8 g NaOH, 350 mg sodium octyl sulphate, 80 mg disodium EDTA, 1 ml triethylamine, 6% MeOH, 6% CH_3CN per litre of water (final pH = 5) was pumped at a rate of $60\,\mu l\,min^{-1}$ with a constant column pressure of 2500–3000 psi. Standard curves of DA, constructed before the injection of dialysate samples, were linear over a wide range of concentrations. The lowest limit of assay sensitivity for DA was 200 femtograms per 5 μl sample.

Reagents and drugs

The oligonucleotides used for the antisense strategy were from Genosys (The Woodlands, U.S.A.). DOTAP was from Boheringer-Mannheim (Mannheim, Germany). Amphetamine sulphate (AMPH), benzylamine hydrochloride (BZ) and 4-MBZ were purchased from the Sigma Chemical Company (St Louis, MO, U.S.A.). Chromatographic reagents, buffer salts and other chemicals used in the microdialysis experiments were obtained from the Sigma Chemical Company (St Louis, MO, U.S.A.). 4-MBZ was administered as hydrochloride obtained by adding to a solution of free base, under pH meter, a suitable amount of 1 M HCl. All the drugs used for the behavioural experiments were dissolved in isotonic (NaCl 0.9%) saline. Dilutions of the compounds were prepared in such a way that the necessary dose could be administered by i.c.v. injection in a volume of 5 μl per mouse. Before i.c.v. administration, it was assessed that the pH values of the nM compound solutions (ranging from 7.2 to 6.7) did not vary significantly from those of the saline ($pH = 6.8 \pm 0.4$). Antisense and degenerated oligonucleotides were dissolved in the vector (DOTAP) at least 30 min before injection.

Statistical analysis

All experimental results are given as the mean ± s.e.m. An analysis of variance (ANOVA) was used to verify significance between two means of the behavioural results, and was followed by Fisher's protected least significant difference procedure for *post hoc* comparison; *P*-values <0.05 were considered significant. Data were analysed using the stat view software for Macintosh (1992). For the microdialysis experiments, the first three samples collected before any experiment were considered as baseline samples; subsequent DA concentrations were expressed as a percentage of the mean of this baseline. For these studies, according to Baumann *et al*. (2000), ANOVA evaluations were performed on the AMPH-, BZ- or 4-MBZ-stimulated percent of DA release and in rats coadministered with AMPH and 4-MBZ in comparison with the basal release of controls.

Results

In mice deprived of food for only 4 h and therefore slightly stimulated to eat (only $227 \pm 10\,mg\,mouse^{-1}\,h^{-1}$ of food was consumed by 4-h fasting controls, as compared to $665 \pm 9\,mg\,mouse^{-1}\,h^{-1}$; $n = 10$, $P < 0.01$, after 12 h of fasting), AMPH (2 μg) and BZ (33 μg; 231 nmol) still retained a weak, but not significant, hypophagic response by reducing the food intake of 12 and 8%, respectively, of the controls. On the contrary, 4-MBZ (1.2–54.8 μg; 10–452 nmol) dose dependently stimulated food consumption (Figure 1a), with an increase of about 190% in the food consumed by the controls and an ED_{50} of $8.3 \pm 2.7\,\mu g\,mouse^{-1}$ ($68 \pm 22\,nmol\,mouse^{-1}$). Hyperphagic behaviour was also elicited by 4-MBZ after i.p. administration ($10–600\,mg\,kg^{-1}$), with a maximum increase in food ingestion of about 160% over saline-treated controls and an approximate ED_{50} of $129\,mg\,kg^{-1}$ (Figure 1b).

In 4-h fasting mice, the 4-MBZ-induced hyperphagic response (28 μg; 231 nmol) (Figure 2) was dose dependently reduced by pretreatments with $aODN_1$ (3, 6, 9 nmol of ODNs on days 1, 4, 7 before food intake experiments), whereas $dODN_1$, which was unable to modify the expression of Kv1.1 channels and was therefore used as negative control, was without effect. At the time selected for the food consumption experiments (48 h after the last injection of the ODNs), it was

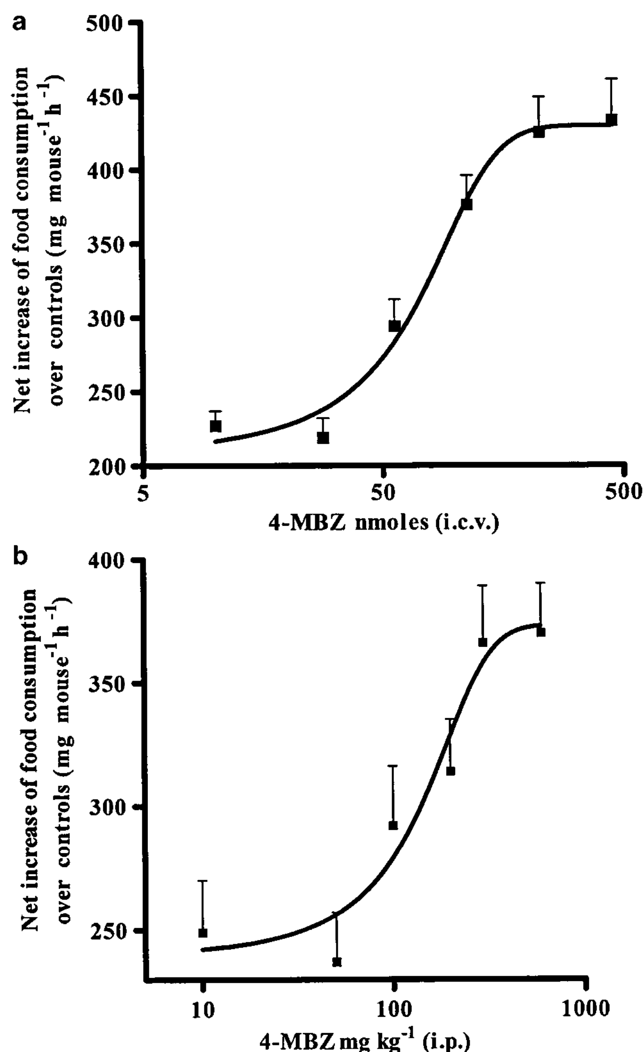

Figure 1 Effect of increasing doses of 4-MBZ on the food intake in 4-h fasting mice 60 min after food readministration: (a) 4-MBZ-induced increase of food intake over controls after i.c.v. administration or (b) 4-MBZ-induced increase of food intake over controls after i.p. administration in mice. Each point represents the mean ± s.e.m. of at least 10 mice.

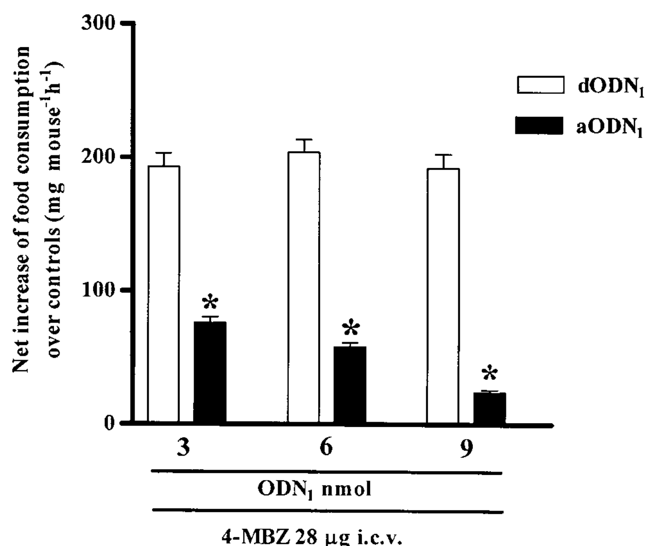

Figure 2 Effect of aODN$_1$ or dODN$_1$ pretreatments (3, 6, 9, nmol per single i.c.v. injection at days 1, 4, 7) on the food intake increase induced by 28 μg (231 nmol) of i.c.v.-injected 4-MBZ in 4-h fasting mice, 60 min after food readministration. *$P<0.01$ in comparison with dODN$_1$-pretreated mice taken as controls. Each point represents the mean ± s.e.m. of at least 10 mice. One-way ANOVA also showed a value of $P<0.01$ level of significance among the three groups receiving different doses of aODN$_1$ pretreatments.

also verified that neither aODN$_1$ nor dODN$_1$ alone induced any significant variation in the food consumption of pretreated animals as compared with control, saline-injected mice. In particular, the food consumption in 3, 6, 9 nmol of aODN$_1$-pretreated mice was, respectively, of 238 ± 18, 228 ± 17, 215 ± 17, whereas the corresponding values in dODN$_1$-pretreated animals were 249 ± 22, 236 ± 22, 244 ± 22 mg mouse^{-1} h^{-1}. These values did not differ significantly from the food consumption of 231 ± 9 mg mouse^{-1} h^{-1} as measured in saline-injected animals.

At the experimental settings of 6 and 9 nmol of aODN$_1$ pretreatment, the percentage of reduction of mRNA levels for Kv1.1 was not measured; however, a significant reduction ($P<0.01$) of 4-MBZ-induced hyperphagic response (28 μg; 231 nmol) over dODN$_1$-treated controls was obtained. Instead, because the dose of 3 nmol of aODN$_1$ was already demonstrated to reduce mRNA levels (Ghelardini *et al.*, 1997), this dose was used for all the further experiments. In 3, 6, 9 nmol dODN$_1$-pretreated mice, a food ingestion of 193 ± 10, 204 ± 9 and 192 ± 10 mg mouse^{-1} h^{-1} was obtained, while the amount of food ingested by mice after 3, 6, 9 nmol of aODN$_1$ pretreatments was 76 ± 4, 58 ± 3 and 24 ± 2 mg mouse^{-1} h^{-1}, respectively, resulting in an inhibition of 60, 71, 89%, respectively. Moreover, a significant reduction of food intake among the three different groups of aODN$_1$ treatment was also obtained ($P<0.01$).

In other experiments, performed in 4-h fasting mice, we found that equiactive doses of AMPH (2 μg) or BZ (33 μg; 231 nmol) significantly reduced the hyperphagic response of coadministered 4-MBZ (28 μg; 231 nmol) to about 69.6 and 44.9%, respectively (Figure 3a). Conversely, the hypophagic activity of AMPH (2 μg) or BZ (33 μg; 231 nmol), in 12-h fasting mice contemporaneously receiving 4-MBZ (28 μg; 231 nmol), was decreased to about 73.9 and 72.6% (Figure 3b), respectively. Furthermore, a significant antagonism of AMPH-stimulated motility and inspection activity on the hole-board test (Table 1) was also observed for 4-MBZ which was devoid *per se* of any significant effect on mice as compared with the saline-injected controls.

Microdialysis experiments, in freely moving rats, also showed that, expressed as percentage change from controls, 120–160 min after BZ (33 μg; 231 nmol) or AMPH (2 μg) infusion, the maximum increase in DA efflux from nucleus accumbens (60–80% over the basal values) was observed in rats (Figure 4a and b). No changes over the basal efflux of DA was induced by 4-MBZ (28 μg; 231 nmol) administered alone to the animals (Figure 4c), whereas this compound significantly reduced the AMPH-induced DA efflux (Figure 4d).

Discussion

4-MBZ elicits hyperphagic activity in mice, which is the opposite of the hypophagic effect of the parent compound BZ

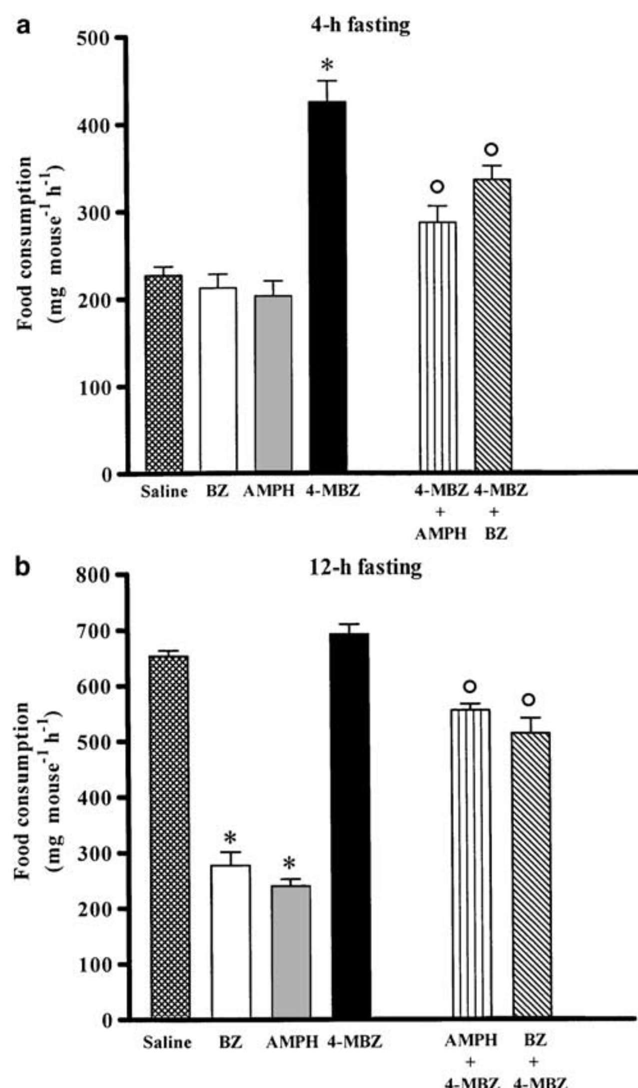

Figure 3 Inhibition induced by (a) AMPH (2 μg) and BZ 33 μg (231 nmol) on the hyperphagic effect of 4-MBZ 28 μg (231 nmol) in 4 h fasted mice; the compounds were coadministered i.c.v. in 5 μl of saline. $^*P<0.01$ in comparison with saline-injected controls; $^\circ P<0.01$ in comparison with the hyperphagic effect of 4-MBZ 28 μg. Inhibition induced by (b) 4-MBZ 28 μg on the hypophagic effect AMPH (2 μg) and BZ 33 μg in 12 h fasted mice; the compounds were coadministered i.c.v. in 5 μl of saline. $^*P<0.01$ in comparison with saline-injected controls; $^\circ P<0.01$ in comparison with the hypophagic effect of AMPH or BZ. Each point represents the mean $\pm$ s.e.m. of at least 10 mice.

Table 1 Effect of AMPH, 4-MBZ, AMPH + 4-MBZ, on hole-board test in mice

Treatment (i.c.v.)	*Hole-board test No. of movements on the plane*	*No. of head plunging*
Saline (5 μl)	31.5 $\pm$ 4.3	21.8 $\pm$ 3.3
4-MBZ (28 μg)	33.4 $\pm$ 4.9	20.6 $\pm$ 4.0
AMPH (2 μg)	71.5 $\pm$ 7.7*	34.2 $\pm$ 5.1**
4-MBZ (28 μg) + AMPH (2 μg)	42.6 $\pm$ 6.5°	23.5 $\pm$ 3.7°

$^{**}P<0.05$; $^*P<0.01$ in comparison with saline-treated mice. $^\circ P<0.01$ in comparison with 2 μg AMPH-treated mice. The values are the mean $\pm$ s.e.m. of at least 10 mice per group.

or that of AMPH. The present results, which were obtained in mice not maximally stimulated to eat (4-h fasting), show that i.c.v.- or i.p.-administered 4-MBZ dose dependently increases food consumption, thus confirming that the simple methyl substitution in position 4 of the benzene ring inverts the anorexigenic property of BZ. This feature, which is not present in 2- or 3-alkyl or halogen derivatives nor in alkyl-amino substitutes of BZ (Raimondi *et al.*, 2003), suggests the utility of more detailed SAR studies in order to find the best alkyl substitution for this effect.

The results obtained after i.c.v. administrations in mice show that the hyperphagic effect of 4-MBZ is dose dependently reduced by $aODN_1$, from about 60 to 89%, as compared with that of control $dODN_1$-pretreated animals. Similar $aODN_1$ pretreatments were previously found to decrease by more than 50% the *Shaker-like* Kv1.1 mRNA channel expression in the brain (Galeotti *et al.*, 1997a, b) and almost completely abolish the hypophagic responses induced by AMPH and BZ in 12-h fasting mice (Banchelli *et al.*, 2001; Pirisino *et al.*, 2001). Therefore, it appears that 4-MBZ, as well as AMPH and BZ, to exert their opposite effects on food consumption in mice, require the complete expression of the *Shaker-like* Kv1.1 channels. Conversely, the food consumption of control mice starved for 4 or 12 h is not influenced at the same experimental condition, suggesting that just the 50% reduction of Kv1.1 expression is enough to maintain the physiological response. These results confirm our previous observations on the role of potassium channels in alimentary behaviour and the observation that *Shaker-like* Kv1.1 subtypes play a role in the central stimulating effects of AMPH.

It is known that potassium channels, by regulating the action potential in neurons, modulate the extracellular concentrations of DA or 5-HT known to be involved in alimentary behaviour (Cook & Quast, 1990; Boireau *et al.*, 1991; Dawson & Routledge, 1995; Inui, 2000). Some electrophysiological results also indicate that AMPH and related compounds may block K^+ currents in different tissues (Hu *et al.*, 1998; Casis *et al.*, 2000) and it has been found that K^+ channels openers, injected i.c.v. in mice, increase food intake, whereas blockers induce hypophagic effects (Ghelardini *et al.*, 1997). Thus, although direct electrophysiological investigations were outside the scope of the present work, we hypothesize that 4-MBZ – at least at the doses used in our investigations – elicits hyperphagic effects in mice probably by opening *Shaker-like* Kv1.1 subtypes in the brain, whereas AMPH and BZ could induce hypophagic effects by blocking these channels. This conclusion could also explain the results of experiments in which both the hyperphagic responses induced by 4-MBZ and the hypophagic effects elicited by AMPH and BZ were studied, respectively, in 4- or in 12 h-fasting mice coadministered i.c.v. with these compounds: 28 μg of 4-MBZ were almost equipotent to 2 μg of AMPH or 33 μg of BZ in reciprocally counteracting their own pharmacological properties. The hypophagic effect of AMPH and BZ in 4 h-fasted mice was measurable but it was not statistically significant (Figure 3a). This can result from the fact that, after 4 h of fasting, mice consumed a very low amount of food that was only weakly modified by hypophagic treatments.

AMPH also elicits motor stimulatory effects, mainly by increasing the extracellular concentration of DA, 5-HT or other neurotransmitters in the brain, the nucleus accumbens being an important target for this effect (Giros *et al.*, 1996;

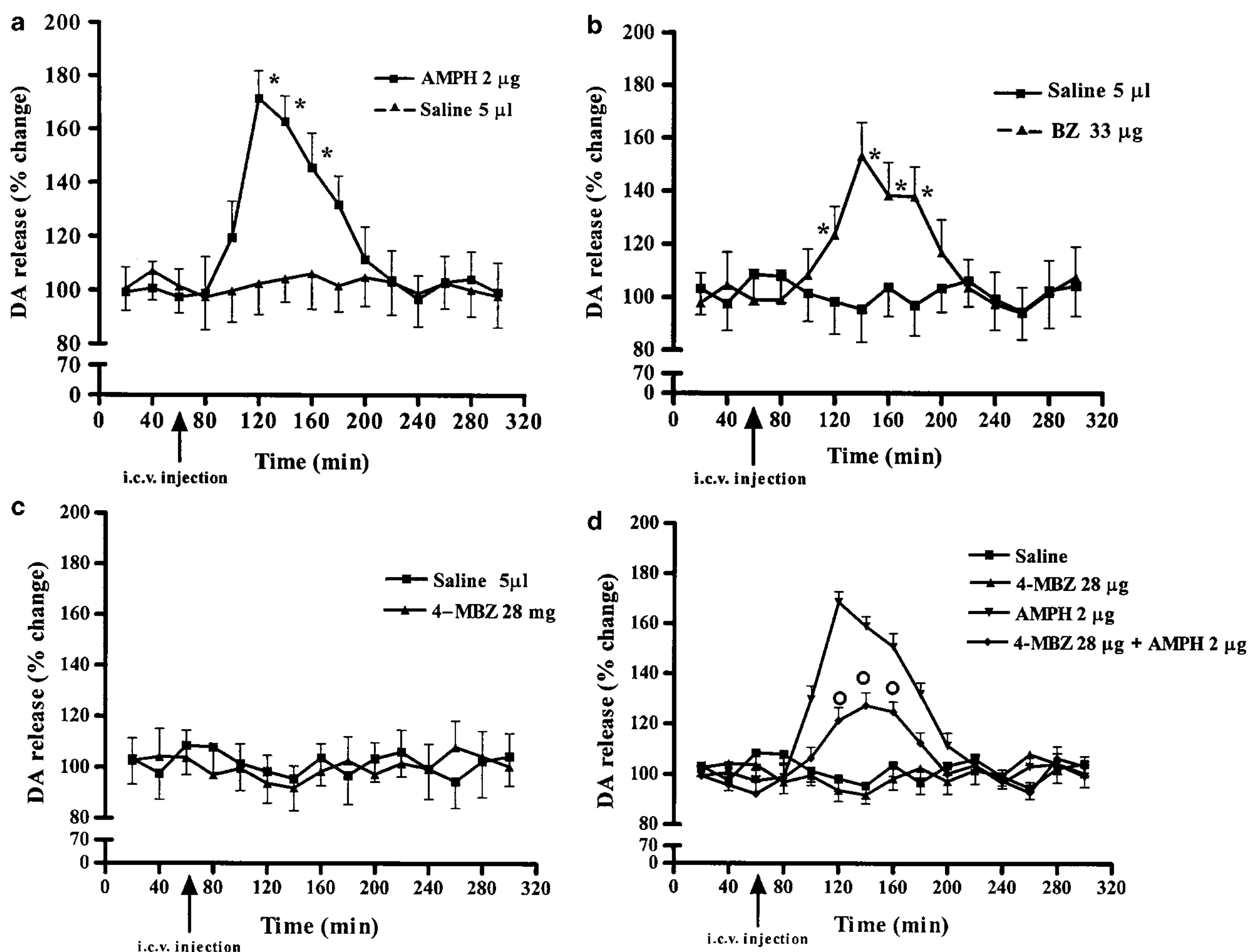

Figure 4 The effect of (a) 2 μg i.c.v. AMPH, (b) 28 μg (231 nmol) i.c.v. 4-MBZ, (c) 33 μg (231 nmol) i.c.v. BZ, (d) 28 μg i.c.v. 4-MBZ + 2 μg i.c.v. AMPH on DA release from the nucleus accumbens in freely moving rat. *$P<0.01$ in comparison with the values obtained at the same times in saline, (5 μl) injected, controls. °$P<0.01$ in comparison with the values obtained at the same times in 2 μg AMPH-treated rats. Each point represents the mean ± s.e.m. of five rats.

Bardo, 1998; Chiamulera *et al.*, 2001). Microdialysis investigations have recently shown that aODN$_1$ pretreatments significantly reduced the DA efflux elicited by AMPH from the nucleus accumbens of rats (Ghelardini *et al.*, 2003). Furthermore, some results of the present investigations (not shown) indicate that 2 μg AMPH, given i.c.v., failed to increase the basal release of 5-HT in this brain area. These observations are in agreement with those of (Baumann *et al.*, 2000) showing that doses of AMPH below 10 μg i.c.v. increased the extracellular DA, but not 5-HT levels, in the nucleus accumbens. Our results also indicate that, like aODN$_1$, 4-MBZ counteracts the stimulation of DA release induced by AMPH and BZ.

Together with the hypophagic response, aODN$_1$ pretreatments were also found to reduce other neurobehavioural effects of AMPH, including hypermotility, in mice (Ghelardini *et al.*, 2003). Again, in our experiments, 4-MBZ reduced the motor stimulatory effects as well as the increase of inspection activity induced by AMPH, showing properties similar to that of the potassium channels openers, also reported to attenuate the hyperlocomotory effects of AMPH (Rosenzweig-Lipson *et al.*, 1997). The ability of 4-MBZ to counteract the AMPH-induced central stimulation in mice was also verified in AMPH-induced reduction in sleeping time or in AMPH-induced seizures (not shown), further indicating that the pharmacological profile of this compound is very similar to that already described for aODN$_1$ (Ghelardini *et al.*, 2003). It is appropriate to point out that, in all the behavioural experiments we have performed, the administration of 4-MBZ alone did not change the basal parameters of the animals, indicating that this compound did not show, at least at concentrations used in these experiments, any relevant interference in the gross activity of the animals.

Conclusions

The reduction of *Shaker-like* Kv1.1 potassium channel expression, by i.c.v.-administered aODN$_1$, was previously described to abolish the most relevant stimulatory effects of AMPH. We describe here a novel compound, 4-MBZ, capable like aODN$_1$ of counteracting hypophagia, hypermotility and neurochemical dopaminergic activity of AMPH acting on Kv1.1 subtypes. This compound, suitable for systemic administration, could be of potential interest as an antidote of central pharmacotoxicological effects of AMPH.

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