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## Effect of K<sup>+</sup> channel modulation on mouse feeding behaviour

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### Abstract

The K<sup>+</sup> channel antagonists, glucose (100 µg per mouse i.c.v.), tetraethylammonium (1 µg per mouse i.c.v.) and apamin (1 ng per mouse i.c.v.), reduced food intake of mice comparably to the two anorectic drugs, amphetamine (10 µg per mouse i.c.v.) and cocaine (50 µg per mouse i.c.v.). Conversely, the K<sup>+</sup> channel openers, minoxidil (5 µg per mouse i.c.v.) and pinacidil (10 µg per mouse i.c.v.), elicited an orectic effect of the same intensity as that induced by 2-deoxyglucose (200 µg per mouse i.c.v.), aurothioglucose (200 µg per mouse i.c.v.) and neuropeptide Y (0.5 µg per mouse i.c.v.). The antisense oligodeoxyribonucleotide (1–3 nmol per injection) to mKv1.1 gene produced, at 72 h, a dose-dependent increase in food intake. A quantitative reverse transcription-polymerase chain reaction (RT-PCR) study demonstrated a reduction in cerebral mRNA levels only in the antisense oligodeoxyribonucleotide-treated group, indicating the absence of a sequence-independent action. Mice receiving the K<sup>+</sup> channel modulators or antisense oligodeoxyribonucleotide had unmodified motor coordination and inspection activity as revealed, respectively, by the rotarod and hole-board tests. The integrity and functionality of central K<sup>+</sup> channels appears, therefore, to be fundamental in the regulation of food intake by mice. © 1997 Elsevier Science B.V.

**Keywords:** K<sup>+</sup> channel; Antisense oligodeoxyribonucleotide; Food intake; K<sup>+</sup> channel opener; K<sup>+</sup> channel antagonist

### 1. Introduction

A variety of substances have been demonstrated to increase (α-adrenoceptor agonists, dopamine, opioid peptides, neuropeptide Y, muscimol, acetylcholine, benzodiazepines, etc.) or decrease (epinephrine, β-adrenoceptor agonists, dopamine, serotonin, corticotrophic releasing factor, thyrotropin-releasing hormone, bombesin, calcitonin, calcitonin gene-related peptide, cholecystokinin, insulin, etc.) feeding after their central administration (Morley and Levine, 1985).

The central regulation of food consumption is controlled by the hypothalamic cells (Morley, 1980). Electrophysiological studies have demonstrated that some neurones located in this cerebral area are stimulated by the enhancement of extracellular levels of glucose (Ono et al., 1982; Minami et al., 1986). Different types of neurones sensitive to glucose have been identified in the guinea-pig ventromedial hypothalamic area (Minami et al., 1986).

Furthermore, reduced action potential has been observed as a consequence of glucose injection into the rat lateral hypothalamus (Oomura et al., 1969).

Ashford et al. (1990a,b,c) and Boden et al. (1989) have reported that the increase in the frequency of hypothalamic neuronal spiking in rats that follows augmented plasma glucose levels depends on the inactivation of ATP-dependent K<sup>+</sup> channels (K<sub>ATP</sub> channels).

On the basis of results of the above-mentioned electrophysiological studies, we thought it worthwhile to investigate the role of K<sub>ATP</sub> channels in the regulation of food consumption in mice and to extend the research to the voltage-dependent K<sup>+</sup> channels. To this end both a pharmacological and an antisense strategy were used. A specific antisense oligodeoxyribonucleotide, targeting the translation start region of the mKv1.1 mRNA, was designed. Antisense oligodeoxyribonucleotides are short synthetic DNA segments complementary to sequences of an mRNA target. By forming DNA/mRNA heteroduplexes, antisense oligodeoxyribonucleotides can transiently inactivate single genes and are, therefore, used both for studies of gene expression and as potential informational drugs (Wahlestedt, 1994). The effect of intracerebroventricular

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injection of K<sup>+</sup> channel openers (minoxidil and pinacidil), K<sup>+</sup> channel antagonists (glucose, apamin and tetraethylammonium) and the mKv1.1 antisense oligodeoxyribonucleotide was evaluated.

## 2. Materials and methods

### 2.1. Animals

Male Swiss albino mice (23–30 g) from Morini (San Polo d'Enza, Italy) were used. The mice were housed 15 per cage. The cages were placed in the experimental room 24 h before the test for adaptation. The animals were kept at 23 ± 1°C with a 12 h light/dark cycle, light on at 7 a.m.

### 2.2. Antisense oligonucleotides

The 24mer phosphodiester oligonucleotides were capped by a terminal phosphorothioate double substitution and purified by chromatography (Genosys, The Woodlands, TX, USA). The antisense oligodeoxyribonucleotide (5'-CGA CAT CAC CGT CAT GAT GAA AGC-3') was designed targeting the 5' portion of the murine Kv1.1 (mKv1.1) mRNA, residues 575–598 of the published cDNA sequence (Chandy et al., 1990). A fully degenerated 24mer oligodeoxyribonucleotide was used as control.

### 2.3. Administration of antisense oligonucleotide

Mice were randomly assigned to an antisense oligodeoxyribonucleotide, degenerated oligodeoxyribonucleotide or vector group. 100, 200 and 600 µM oligodeoxyribonucleotides were preincubated at 37°C for 30 min with 13 µM DOTAP (*N*-[1-(2,3-dioleoyloxy)propyl]-*N,N,N*-trimethylammonium methyl sulfate), used as vector. Each group received a single intracerebroventricular (i.c.v.) injection on days 1, 4 and 7. The behavioural tests were performed 72 h after the last i.c.v. injection of vector or oligodeoxyribonucleotides.

### 2.4. Administration of drugs

Mice were randomly assigned to a control (saline solution) or a treated (aurothioglucose, 2-deoxyglucose, neuropeptide Y, glucose, minoxidil, pinacidil, amphetamine, cocaine, apamin, tetraethylammonium) group. The mice were i.c.v. injected with saline or a drug 5 min before the evaluation of food consumption.

### 2.5. Intracerebroventricular injection technique

Intracerebroventricular administration was performed under ether anaesthesia using isotonic saline as a solvent,

according to the method described by Haley and McCormick (1957). Briefly, during anaesthesia, mice were grasped firmly by the loose skin behind the head. A 0.4 mm external diameter, hypodermic needle 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 5 µl was then administered. The injection site was 1 mm to the right or left from the midpoint on a line drawn through to the anterior base of the ears. Injections were performed into the right or left ventricle randomly. To ascertain that the oligodeoxyribonucleotides were administered exactly into the cerebral ventricle, some mice were injected with 5 µl of 1:10 diluted India ink and their brains were examined macroscopically after sectioning.

### 2.6. Behavioural tests

#### 2.6.1. Evaluation of food consumption

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 weight 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 injection, to an accuracy of 0.1 g. An arbitrary cut-off time of 60 min was used.

#### 2.6.2. Hole-board test

The hole-board test set up consisted of a 40 cm square plane with 16 flush-mounted cylindrical holes (diameter 3 cm) distributed 4 by 4 in an equidistant, grid-like manner. Mice were placed on the center of the board one by one and left to move about freely for a period of 5 min each. Two photoelectric beams, crossing the plane from midpoint to mid-point of opposite sides, thus dividing the plane into 4 equal quadrants, automatically signaled the movement of the animals on the surface of the plane. Miniature photoelectric cells, in each of the 16 holes, recorded the exploration of the holes (head plunging activity) by the mice. The test was performed 30 min after i.c.v. administration of minoxidil and pinacidil, and 60 min after administration of tetraethylammonium.

#### 2.6.3. Rotarod test

The apparatus consisted of a base platform and a rotating rod of 3 cm diameter with a non-skid surface. The rod was placed at a height of 15 cm from the base. The rod, 30 cm in length, was divided into 5 equal sections by 6 disks. Thus up to 5 mice were tested simultaneously on the apparatus, with a rod-rotation speed of 16 r.p.m. The integrity of motor coordination was assessed on the basis of the number of falls from the rod in 30 s according to Vaught et al. (1985). Performance time was measured before and 15, 30 and 45 min after i.c.v. administration of saline or drugs.

## 2.7. In vitro studies

### 2.7.1. RT-PCR-based analysis of *mKv1.1* mRNA

Mouse brain levels of *Kv1.1* mRNA were determined by a quantitative reverse transcription-polymerase chain reaction (RT-PCR) method (Quattrone et al., 1995a,b). Briefly, 72 h after the last i.c.v. injection of vector, antisense oligodeoxyribonucleotide or degenerated oligodeoxyribonucleotide, some mice were killed and their brains were rapidly removed and stored ( $-80^{\circ}\text{C}$ ). Frozen brain samples (0.2–0.3 g wet weight) were homogenised in 3 volumes of RNazol in order to extract total RNA according to the manufacturer's instructions. RNA was treated with RQ1 RNase-free DNase, purified by ethanol precipitation, dissolved in water containing an RNase inhibitor (RNasin at 1 U/ml) and reversely transcribed to cDNA using random hexamers. *Kv1.1* amplimers were 5'-GCT CTC TCC TGG CCT CCT-3' and 5'-GTT TCG AAG CGC AGC CCG-3', residues 544–561 and 715–732 respectively, according to the *Kv1.1* cDNA published sequence (Chandy et al., 1990).  $\beta$ -Actin was used as internal standard gene. PCR cycles consisted of 1 min denaturation at  $92^{\circ}\text{C}$ , 1 min annealing at  $56^{\circ}\text{C}$  and 1 min extension at  $72^{\circ}\text{C}$  for 30 cycles. Amplification products were first identified by sequencing then quantified by densitometry.

## 2.8. Reagents and drugs

Oligonucleotides used for the antisense strategy, and specific primers or hexamers used for RT-PCR analysis were from Genosys (The Woodlands, USA). DOTAP was from Boehringer-Mannheim (Mannheim, Germany). RNazol was from Cynna Biotech (Houston, USA); RQ1 RNase-free DNase, RNase ONE, RNasin, Mo-MLV reverse transcriptase and fmol sequencing kit were from Promega (Madison, WI, USA); *Taq* polymerase was from Perkin-Elmer-Cetus (Emeryville, USA). The following drugs were used: aurothioglucose, 2-deoxyglucose (Sigma); neuro peptide Y, minoxidil, pinacidil, tetraethylammonium chloride, apamin (RBI); glucose (Merck); cocaine (U.S.L. 10/D, Florence); D-amphetamine (De Angeli).

Drugs were dissolved in isotonic (NaCl 0.9%) saline solution, with the exception of pinacidil that was dissolved in a water and dimethyl sulfoxide (3:1) vehicle, immediately before use. Antisense and degenerated oligonucleotides were dissolved in the vector (DOTAP) at least 30 min before injection. Drug concentrations were prepared in such a way that the necessary dose could be administered in a volume of 5  $\mu\text{l}$  per mouse by i.c.v. injection.

## 2.9. Sequence and statistical analysis

Sequences of *Kv* genes are from the GenBank database. Sequence comparisons of both antisense oligodeoxyribonucleotide and RT-PCR primers with the database were carried out using the FASTA program. All experimental

results are given as the means  $\pm$  S.E.M. Analysis of variance (ANOVA), followed by Fisher's protected least significant difference (PLSD) procedure for post-hoc comparison, was used to verify the significance of differences between two means of behavioural results. Data were analysed with the StatView software for the Macintosh (1992). The statistical significance of RT-PCR was obtained using Student's *t*-test; *P* values of less than 0.05 were considered significant.

## 3. Results

### 3.1. Food intake

The cumulated amount of food eaten by mice not deprived of food was compared with that eaten by mice which had no access to food for 4 or 12 h before the test (Fig. 1). The level of food intake progressively increased up to 45 min ( $240 \pm 14.1$  mg per mouse) in the 4-h food-deprived mice and then remained almost unchanged. The 12-h food-deprived mice had a constant increase in the amount of food consumed in a 60 min test ( $630 \pm 10$  mg per mouse) (Fig. 1).

Glucose (100  $\mu\text{g}$  per mouse i.c.v.), apamin (1 ng per mouse i.c.v.) and tetraethylammonium (1  $\mu\text{g}$  per mouse i.c.v.) significantly reduced food consumption in 12-h food-deprived mice (Fig. 2). The anorectic effect produced by the above-mentioned drugs was comparable to that exerted by amphetamine (1 and 10  $\mu\text{g}$  per mouse i.c.v.) and cocaine (1 and 50  $\mu\text{g}$  per mouse i.c.v.), used as

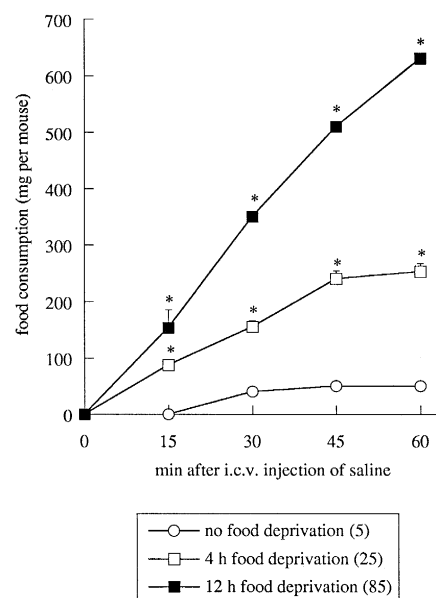


Fig. 1. Time-course of cumulated food intake in mice after different times (0, 4 and 12 h) of food deprivation. Mice were i.c.v. injected with saline solution 5 min before the test. Vertical lines represent S.E.M.; in parentheses the number of mice is reported. \*  $P < 0.001$  in comparison with mice not food-deprived.

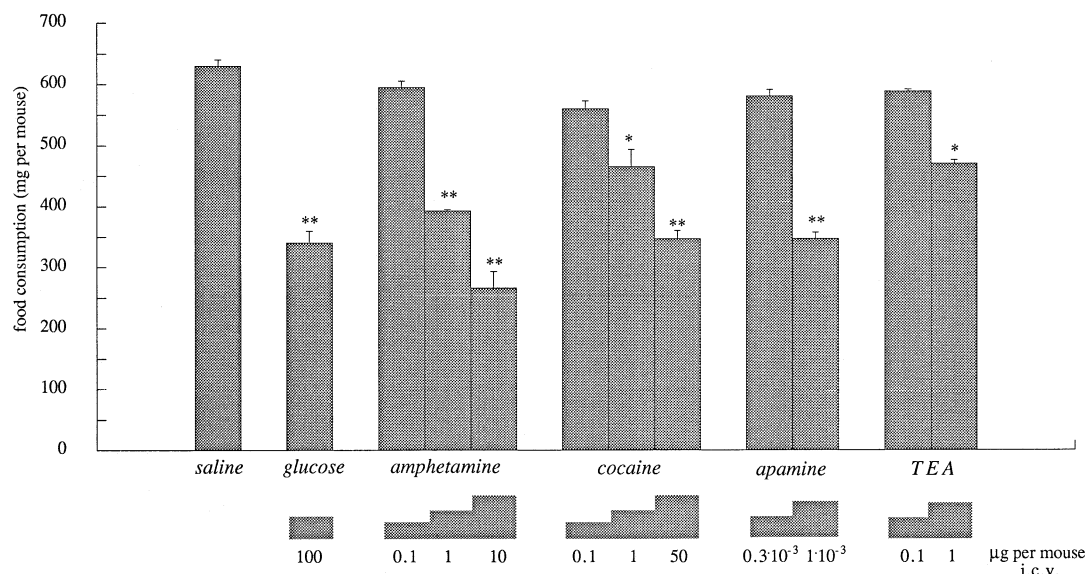


Fig. 2. Dose-dependent suppression of food intake of 12-h food-deprived mice by glucose, apamin and tetraethylammonium (TEA) in comparison with amphetamine and cocaine. Mice were i.c.v. injected with saline solution or a drug 5 min before the test. The food intake values were evaluated as the cumulated amount of food eaten 60 min after the beginning of the test. Vertical lines represent S.E.M.; the number of mice was between 10 and 20 with the exception of saline-treated mice ( $n = 85$ ). \*  $P < 0.01$ , \*\*  $P < 0.001$  in comparison with saline-treated mice.

reference drugs (Fig. 2). The food intake values reported in Fig. 2 were evaluated as the cumulated amount of food eaten 60 min after the beginning of the test.

Fig. 3 shows the orectic effect of the two  $K^+$  channel openers minoxidil (5 µg per mouse i.c.v.) and pinacidil (1 and 10 µg per mouse i.c.v.) in mice which were not provided with food for 4 h. This increase in food consumption was compared with that produced by 2-deoxyglucose (200 µg per mouse i.c.v.) and aurothioglucose (200 µg per mouse i.c.v.) which were used as reference drugs.

Repeated administration of an antisense oligodeoxyribonucleotide to the mKv1.1 gene produced a dose-response increase in food consumption by mice after 4 h of food deprivation, as illustrated in Fig. 4. The dose of antisense oligodeoxyribonucleotide of 0.5 nmol per i.c.v. injection was devoid of any orectic effect while the dose of 1 and 3 nmol per i.c.v. injection increased mouse food intake by, respectively, 82 and 200% in comparison with vector-treated mice (Fig. 4). The degenerated oligodeoxyribonucleotide did not modify the amount of

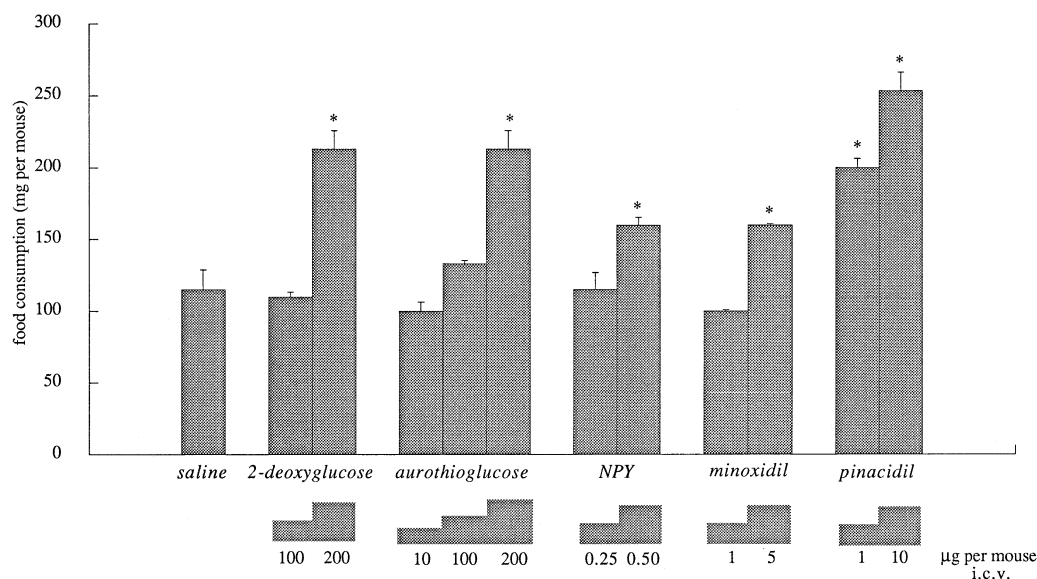


Fig. 3. Dose-dependent increase in food intake by mice deprived of food for 4 h after administration of minoxidil and pinacidil in comparison with 2-deoxyglucose, aurothioglucose and neuropeptide Y. Mice were i.c.v. injected with saline solution or a drug 5 min before the test. The food intake values were evaluated as the cumulated amount of food eaten 30 min after the beginning of the test. Vertical lines represent S.E.M.; the number of mice was between 10 and 30. \*  $P < 0.001$  in comparison with saline-treated mice.

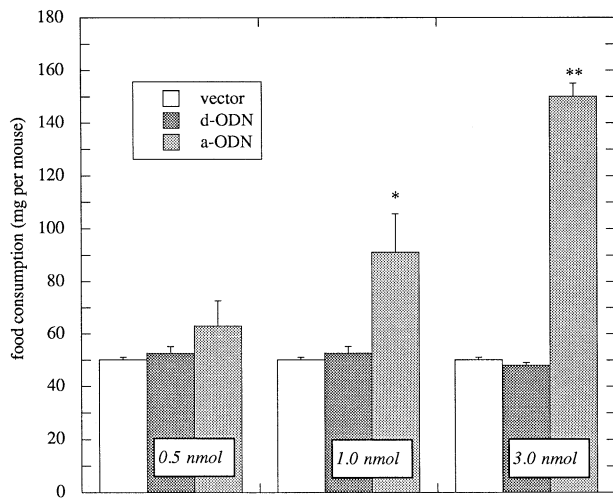


Fig. 4. Dose-dependent increase in food intake of mice deprived of food for 4 h after antisense oligodeoxyribonucleotide (aODN) treatment in comparison with that of degenerated oligodeoxyribonucleotide (dODN) and vector groups. Mice received a single i.c.v. injection on days 1, 4 and 7. The test was performed 72 h after the last i.c.v. injection. The food intake values were evaluated as the amount of food eaten 15 min after the beginning of the test. Vertical lines represent S.E.M.; the number of mice was between 10 and 20. \*  $P < 0.01$ , \*  $P < 0.001$  in comparison with vector-treated mice.

food consumed compared to that of the control mice (Fig. 4).

The food intake values reported in Figs. 3 and 4 corresponded with the maximum effect of minoxidil, pinacidil and mKv1.1 antisense oligodeoxyribonucleotide that occurred respectively 30 min (minoxidil, pinacidil) and 15 min (antisense oligodeoxyribonucleotide) after the beginning of the test.

### 3.2. Effect of treatments on mouse behaviour

The motor coordination of mice treated with glucose (100  $\mu$ g per mouse i.c.v.), apamin (0.001  $\mu$ g per mouse

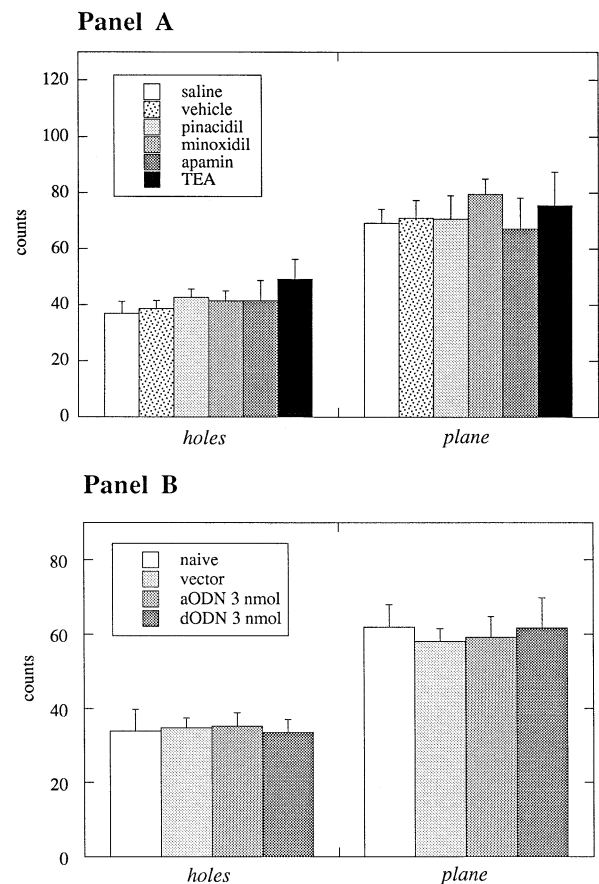


Fig. 5. Lack of effect of  $K^+$  channel antagonists, apamin (1 ng per mouse i.c.v.) and tetraethylammonium (TEA, 1  $\mu$ g per mouse i.c.v.),  $K^+$  channel openers, minoxidil (5  $\mu$ g per mouse i.c.v.) and pinacidil (10  $\mu$ g per mouse i.c.v.) (panel A) and an antisense oligodeoxyribonucleotide to mKv1.1 gene (panel B) in the mouse hole board test. Tetraethylammonium (TEA), apamin and minoxidil were dissolved in saline solution. Pinacidil was dissolved in a  $H_2O$ /DMSO 3:1 vehicle. Antisense oligodeoxyribonucleotide (aODN) and degenerated oligodeoxyribonucleotide (dODN) were dissolved in a vector (DOTAP). The number of mice was between 10 and 16. Vertical lines give S.E.M.

Table 1

Lack of effect of  $K^+$  channel antagonists (apamin and tetraethylammonium),  $K^+$  channel openers (minoxidil and pinacidil) and an antisense oligodeoxyribonucleotide (ODN) to mKv1.1 gene in the mouse rotarod test

Treatment i.c.v.	Number of falls in 30 s			
	Pre-test	After the beginning of the test		
		15 min	30 min	45 min
Saline	2.8 $\pm$ 0.4	2.0 $\pm$ 0.5	1.6 $\pm$ 0.2	0.8 $\pm$ 0.3
Vehicle	2.5 $\pm$ 0.2	1.7 $\pm$ 0.4	1.6 $\pm$ 0.5	1.1 $\pm$ 0.2
TEA 1 $\mu$ g	2.7 $\pm$ 0.3	1.7 $\pm$ 0.6	1.1 $\pm$ 0.2	0.5 $\pm$ 0.2
Apamin 0.1 ng	3.6 $\pm$ 0.3	2.0 $\pm$ 0.5	1.0 $\pm$ 0.4	0.8 $\pm$ 0.4
Pinacidil 10 $\mu$ g	2.5 $\pm$ 0.4	2.2 $\pm$ 0.4	1.7 $\pm$ 0.6	1.2 $\pm$ 0.3
Minoxidil 10 $\mu$ g	2.6 $\pm$ 0.3	2.0 $\pm$ 0.4	1.1 $\pm$ 0.3	1.0 $\pm$ 0.2
Vector	2.9 $\pm$ 0.5	1.9 $\pm$ 0.4	1.1 $\pm$ 0.4	0.8 $\pm$ 0.4
aODN 3 nmol	3.4 $\pm$ 0.5	1.9 $\pm$ 0.5	1.2 $\pm$ 0.4	0.7 $\pm$ 0.3
dODN 3 nmol	3.0 $\pm$ 0.5	2.0 $\pm$ 0.4	1.4 $\pm$ 0.4	1.0 $\pm$ 0.4

Tetraethylammonium (TEA), apamin and minoxidil were dissolved in saline solution. Pinacidil was dissolved in a 3:1  $H_2O$ /DMSO vehicle. Antisense ODN (aODN) and degenerated ODN (dODN) were dissolved in a vector (DOTAP). The number of mice was between 5 and 10.

i.c.v.), tetraethylammonium (1  $\mu\text{g}$  per mouse i.c.v.), minoxidil (10  $\mu\text{g}$  per mouse i.c.v.), pinacidil (10  $\mu\text{g}$  per mouse i.c.v.), antisense oligodeoxyribonucleotide (3.0 nmol per i.c.v. injection) or degenerated oligodeoxyribonucleotide (3.0 nmol per i.c.v. injection) was evaluated by using the rotarod test performed starting from 15 min after the i.c.v. injection of  $\text{K}^+$  channel modulators or 72 h after the end of the antisense oligodeoxyribonucleotide treatment. The motor coordination of mice treated with both potassium channel openers and antagonists or with antisense oligodeoxyribonucleotide was not impaired in comparison with that of control mice, since each group progressively reduced its number of falls (Table 1), the mice having learned how to balance on the rotating rod.

The spontaneous motility and inspection activity of mice was not modified by the above-mentioned treatments, as revealed by results of the hole-board test performed 30 min after the i.c.v. injection of minoxidil and pinacidil, 60 min after tetraethylammonium and apamin i.c.v. injection, or 72 h after the end of the antisense oligodeoxyribonucleotide treatment (Fig. 5).

### 3.3. Effect of antisense oligodeoxyribonucleotide on specific inhibition of *mKv1.1* gene expression

The lowering of *Kv1.1* mRNA following antisense oligodeoxyribonucleotide administration as an index of *Kv1.1* gene inactivation was quantified by RT-PCR. Quantitative results of *Kv1.1* mRNA brain levels following antisense oligodeoxyribonucleotide treatment confirmed that the observed orectic effect was associated with the specific *Kv1.1* mRNA decrease (Fig. 6).

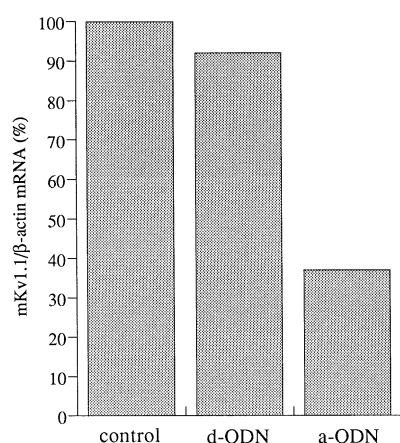


Fig. 6. Quantitative RT-PCR analysis of *mKv1.1* mRNA. Following oligodeoxyribonucleotide (ODN) treatment, total RNA was extracted from the brains of behaviour-tested mice. *mKv1.1* and  $\beta$ -actin mRNA were submitted to RT-PCR as reported in Section 2. Amplification products were analysed on agarose gel and quantified by densitometry. Within the linearity of PCR, the amount of *mKv1.1* mRNA relative to  $\beta$ -actin mRNA was calculated and expressed as percentage of the untreated controls. Data are the means of three determinations.

## 4. Discussion

These studies provide the first reports that the modulation of  $\text{K}^+$  channel activity influences food intake in mice. The administration of  $\text{K}^+$  channel antagonists, such as glucose, apamin and tetraethylammonium, produced a decrease in food consumption. Conversely, the injection of  $\text{K}^+$  channel openers, such as pinacidil and minoxidil, as well as the inhibition of the expression of the *mKv1.1* gene coding for the *Kv1.1*  $\text{K}^+$  channel by using an antisense strategy, increased the amount of food consumed. The orectic or anorectic effect produced by  $\text{K}^+$  channel modulators was of the same magnitude as that produced by well known orectic (2-deoxyglucose, aurothioglucose and neuropeptide Y) and anorectic (amphetamine and cocaine) drugs.

$\text{K}^+$  channel modulators and antisense oligodeoxyribonucleotide, at the doses used in our experiments, did not produce any modification of gross behaviour in mice. The motor coordination, spontaneous motility and inspection activity of treated mice were not impaired in comparison with those of the saline and vector groups, making the results obtained reliable. Higher doses of apamin were not investigated since, starting at the dose of 3 ng per mouse i.c.v., convulsions were elicited. For all other  $\text{K}^+$  channel modulators used, the highest doses reported represent the doses at which the maximum effect was reached.

When considering the described sequence-independent, non-antisense effects of oligodeoxyribonucleotides (Storey et al., 1991; Gao et al., 1992; Blagosklonny and Neckers, 1994; Schick et al., 1995), a fully degenerated phosphodiester-phosphorothioate capped oligodeoxyribonucleotide was used as the most suitable control for these potentially confusing effects. The fully degenerated 24mer is a collection of about  $3 \times 10^{14}$  different molecular species, so that for the nanomolar–micromolar range concentrations achieved in *in vitro* antisense experiments, every species, i.e. every oligodeoxyribonucleotide of defined sequence, was present at the site of action at a sub-attomolar concentration that is totally insufficient to achieve any antisense, or generally sequence-dependent, cellular effect. Therefore, if oligodeoxyribonucleotide i.c.v. administration per se had produced any biological response, this would have been present in degenerated oligodeoxyribonucleotide-treated controls. The degenerated oligodeoxyribonucleotide treatment did not modify mouse food intake in comparison with vector i.c.v. injected mice. This observation ruled out the possibility that the antagonism exerted by antisense oligodeoxyribonucleotide could be due to a sequence-independent action on cerebral structures. The validity of this hypothesis was further supported by results of a quantitative RT-PCR study demonstrating a reduction in *mKv1.1* mRNA levels only in the antisense oligodeoxyribonucleotide-treated group.

All drugs were injected directly into the cerebral ventricles in order to focus the investigation on the central

nervous system and, therefore, to avoid interference from a peripheral mechanism.

The anorectic effect of glucose, apamin and tetraethylammonium was evaluated in mice deprived of food for 12 h. These experimental conditions were needed to better evidence a reduction in food intake. On the other hand, to investigate the orectic effect of minoxidil and pinacidil, mice not deprived of food should be used. Since no significant modification of food consumption was observed in mice having free access to food, food deprivation for 4 h was necessary to evaluate the orectic effect of the two  $K^+$  channel openers.

The possible involvement of  $K_{ATP}$  in the regulation of food intake was postulated based on electrophysiological studies (see Section 1). The present experiments not only confirmed the role of  $K_{ATP}$  in mouse feeding behaviour by means of a behavioural test, but also extended the investigation to the role of other  $K^+$  channel subtypes, such as voltage-dependent  $K^+$  channels, in the modulation of food consumption. The mouse Kv1.1 is a  $K^+$  channel of the *Shaker*-like subfamily that, when expressed in *Xenopus* oocytes, gives rise to a fast-activating, slowly inactivating  $K^+$  current (Hopkins and Tempel, 1992). The investigation into the involvement of mKv1.1 in mouse feeding was carried out because of its wide distribution in the mammalian brain, including areas that are involved in the regulation of food intake (Wang et al., 1994). The expression of the mKv1.1 gene was inhibited by using a specific antisense oligodeoxyribonucleotide and this produced a dose-dependent orectic effect. Antisense oligodeoxyribonucleotides specifically bind to targeted mRNA, preventing translation and/or mediating mRNA cleavage by RNase H and, therefore, down-regulating the synthesis of the encoded protein. Low cell permeability and the high degradation of natural phosphodiester oligomers are considerable drawbacks of the application of antisense oligodeoxyribonucleotides both in vitro and in vivo. In order to overcome these drawbacks, phosphorothioate-capped phosphodiester oligonucleotides, a class of oligodeoxyribonucleotide derivatives that has been shown to maintain more stable and effective concentrations in the brain than do their unmodified counterpart (Whitesell et al., 1993), were used. Furthermore, both oligodeoxyribonucleotide stability and cell uptake are enhanced when oligodeoxyribonucleotides are associated with an artificial cationic lipid (DOTAP) used as an intracellular carrier (Capaccioli et al., 1993; Quattrone et al., 1994).

The anti-mouse Kv1.1 antisense oligodeoxyribonucleotide was designed by comparing the sequence of the translation start sites of mRNAs to the murine Kv1.1 cDNA and other known  $K^+$  channel coding genes, considering that this site is particularly prone to antisense oligodeoxyribonucleotide action (Goodchild, 1989; Stein and Cheng, 1993). The 24-bp segment, 5'-GCT TTC ATC ATG ACG GTG ATG TCG-3' (residues 575–598 of the published mouse Kv1.1 cDNA sequence; Chandy et al.,

1990), has a low sequence homology even with the nearest members of the *Shaker*-like subfamily (54% with Kv1.2, Kv1.3, Kv1.4) and is almost totally unrelated to members of other Kv gene subfamilies. The antisense oligodeoxyribonucleotide used, which is complementary to this Kv1.1 mRNA segment, is therefore most probably unable to target other mouse Kv mRNAs. Moreover, this antisense oligodeoxyribonucleotide has terminal GCs at both the 5'- and 3'-end, known to enhance the binding affinity of antisense oligodeoxyribonucleotide/mRNA heteroduplexes.

The present results not only indicate the possible importance of  $K_{ATP}$  and mKv1.1 in the regulation of food intake of mice, but also confirm the usefulness of antisense strategies for in vivo investigation. Moreover, the similarity between results obtained with antisense oligodeoxyribonucleotide and  $K^+$  channel openers indicates that the lack of the Kv1.1 channel seems to be equivalent to their opening, at least from the orectic point of view.

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## References

- Ashford, M.L.J., Boden, P.R., Treherne, J.M., 1990a. Glucose-induced excitation of hypothalamic neurones is mediated by ATP-sensitive  $K^+$  channels. *Pflüg. Archiv.* 415, 479–483.
- Ashford, M.L.J., Boden, P.R., Treherne, J.M., 1990b. Sulphonylurea excitation of dissociated glucose-responsive hypothalamic neurones in the rat. *J. Physiol.* 420, 93P.
- Ashford, M.L.J., Boden, P.R., Treherne, J.M., 1990c. Tolbutamide excites rat glucoreceptive ventromedial hypothalamic neurones by indirect inhibition of ATP- $K^+$  channels. *Br. J. Pharmacol.* 101, 531–540.
- Blagosklonny, M.V., Neckers, L.M., 1994. Oligonucleotides protect cells from the cytotoxicity of several anti-cancer chemotherapeutic drugs. *Anti-Cancer Drugs* 5, 437–442.
- Boden, P., Ashford, L.J., Treherne, J.M., 1989. Actions of sulphonylureas on neurones of rat ventromedial hypothalamus in vitro. *Br. J. Pharmacol.* 98, 830P.
- Capaccioli, S., Di Pasquale, G., Mini, E., Mazzei, T., Quattrone, A., 1993. Cationic lipids improve antisense oligonucleotide uptake and prevent degradation in cultured cells and in human serum. *Biochem. Biophys. Res. Commun.* 107, 818–825.
- Chandy, K.G., Williams, C.B., Spencer, R.H., Aguilar, B.A., Ghanshani, S., Tempel, B.L., Gutman, G.A., 1990. A family of three mouse potassium channel genes with intronless coding regions. *Science* 247, 973–975.
- Gao, W.-Y., Han, F.-S., Storm, C., Egan, W., Chen, Y.-C., 1992. Phosphorothioate oligonucleotides are inhibitors of human DNA polymerases and RNase H: implications for antisense technology. *Mol. Pharmacol.* 41, 223–229.
- Goodchild, J., 1989. Inhibition of gene expression by oligonucleotides. In: Cohen, J. (Ed.), *Oligodeoxynucleotides, Antisense Inhibitors of Gene Expression*. Macmillan, London, pp. 53–77.



- Haley, T.J., McCormick, G.L., 1957. Pharmacological effects produced by intracerebral injections of drugs in the conscious mouse. *Br. J. Pharmacol. Chemother.* 12, 12–15.
- Hopkins, W.F., Tempel, B.L., 1992. Members of a mouse subfamily of genes encoding voltage-gated potassium channel subunits form heteromultimers when coexpressed in *Xenopus* oocytes. *Soc. Neurosci. Abstr.* 18, 1093.
- Minami, T., Oomura, Y., Sugimori, M., 1986. Electrophysiological properties and glucose responsiveness of guinea-pig ventromedial hypothalamic neurones in vitro. *J. Physiol.* 380, 127–143.
- Morley, J.E., 1980. The neuroendocrine control of appetite: the role of the endogenous opiates, cholecystokinin, TRH, gamma-amino-butyric-acid and the diazepam receptors. *Life Sci.* 27, 355–368.
- Morley, J.E., Levine, A.S., 1985. The pharmacological eating behavior. *Annu. Rev. Pharmacol. Toxicol.* 25, 127–146.
- Ono, T., Nishino, H., Fukuda, M., Sasaki, K., Muramoto, K.-I., Oomura, Y., 1982. Glucoreponsive neurons in rat ventromedial hypothalamic tissue slices in vitro. *Brain Res.* 232, 494–499.
- Oomura, Y., Ono, T., Ooyama, H., Wayner, M.J., 1969. Glucose and osmosensitive neurons of the rat hypothalamus. *Nature* 222, 282–284.
- Quattrone, A., Papucci, L., Schiavone, N., Mini, E., Capaccioli, S., 1994. Intracellular enhancement of intact antisense oligonucleotide steady-state levels by cationic lipids. *Anti-Cancer Drug Design* 9, 549–553.
- Quattrone, A., Fibbi, G., Pucci, M., Anichini, E., Capaccioli, S., Del Rosso, M., 1995a. Reversion of the invasive phenotype of transformed human fibroblasts by antimessenger oligonucleotide inhibiting the urokinase receptor gene expression. *Cancer Res.* 55, 91–95.
- Quattrone, A., Papucci, L., Santini, V., Schiavone, N., Noferini, D., Calastretti, A., Copreni, E., Morelli, S., Rossi Ferrini, P.L., Nicolin, A., Capaccioli, S., 1995b. Quantitation of bcl-2 oncogene expression in cultured follicular lymphoma and primary leukemia B-cells by a highly sensitive RT-PCR method. *Haematologica* 80, 495–504.
- Schick, B.P., Eras, J.L., Mintz, P.L., 1995. Phosphorothioate oligonucleotides cause degradation of secretory but not intracellular serglycin proteoglycan core protein in a sequence-independent manner in human megacaryocytic tumor cells. *Antisense Res. Dev.* 5, 59–65.
- Stein, C.A., Cheng, Y.-C., 1993. Antisense oligonucleotides as therapeutic agents – Is the bullet really magic?. *Science* 261, 1004–1011.
- Storey, A., Oates, D., Banks, L., Crawford, L., Crook, T., 1991. Antisense phosphorothioate oligonucleotides have both specific and non-specific effects on cells containing human papillomavirus type 16. *Nucleic Acids Res.* 19, 4109–4114.
- Vaught, J., Pelley, K., Costa, L.G., Sether, P., Enna, S.J., 1985. A comparison of the antinociceptive responses to GABA-receptor agonists THIP and baclofen. *Neuropharmacology* 24, 211–216.
- Wahlestedt, C., 1994. Antisense oligonucleotide strategies in neuropharmacology. *Trends Pharmacol. Sci.* 15, 42–46.
- Wang, H., Kunkel, D.D., Schwartzkroin, P.A., Tempel, B.L., 1994. Localization of Kv1.1 and Kv1.2, two K channel proteins, to synaptic terminals, somata, and dendrites in the mouse brain. *J. Neurosci.* 14, 4588–4599.
- Whitesell, L., Geselowitz, D., Chavany, C., Fahmy, B., Walbridge, S., Alger, J., Neckers, L.M., 1993. Stability, clearance, and disposition of intraventricularly administered oligodeoxynucleotides: implications for therapeutic application within the central nervous system. *Proc. Natl. Acad. Sci. USA* 90, 4665–4669.