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Methylamine: a new endogenous modulator of neuron firing?

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Summary

Increasing evidence suggests that not only ammonia, but also its alkyl-derivatives, including methylamine, may modulate neuron firing. Methylamine occurs endogenously from amine catabolism and its tissue levels increase in some pathological conditions, including diabetes. Interestingly, methylamine and ammonia levels are reciprocally controlled by a semicarbazide-sensitive amine oxidase activity that deaminates methylamine to formaldehyde with the production of ammonia and hydrogen peroxide. As already described for ammonia, methylamine also targets the voltage-operated neuronal potassium channels, probably inducing release of neurotransmitter(s). From this interaction it has been observed that methylamine is 1) hypophagic in mice without producing amphetamine-like effects and 2) a stimulator of nitric oxide release from rat hypothalamus. Methylamine hypophagia is also maintained in genetically obese and diabetic mice and is increased when these animals are pre-treated with α-amino guanidine, an inhibitor of methylamine oxidative deamination. The effect of α-amino guanidine suggests a potential beneficial effect of this drug, and other such inhibitors, in controlling food intake in animals with disturbed eating behavior. Moreover, the activity of methylamine as an inducer of NO release suggests a role for the amine and for the enzymatic activity that degrades it in neurodegenerative diseases.

key words: **semicarbazide-senstitive amine oxidase activity • methylamine • ammonia • nitric oxide • potassium channels • hypophagia**

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BACKGROUND

Methylamine (MET) is, after ammonia (NH_4^+), the simplest primary amine found in the body which results from endogenous, exogenous, or dietary sources. It is actively metabolized through oxidative deamination carried out by the benzylamine oxidase activity (Bz-SSAO), an enzyme belonging to the semicarbazide-sensitive amine oxidase class (SSAO; EC 1.4.3.6), for which a soluble and a membrane-bound isoform have been described. Membrane-bound Bz-SSAO is highly expressed in adipocytes [1] and smooth muscle cell membranes [2], from which it derives the truncated isoform found circulating in some pathological conditions [3]. Both the membrane-bound and the soluble enzyme share high homology with the vascular adhesion protein VAP-1, a protein involved in lymphocyte-endothelium adhesion [4]. From this, the nomenclature of the enzyme can be updated to Bz-SSAO/VAP-1. The Bz-SSAO/VAP-1 deaminating activity transforms MET to formaldehyde with the production of hydrogen peroxide and NH_4^+. This enzyme exerts a crucial role in controlling the reciprocal levels of NH_4^+ and MET at the site of its catalysis. While the cellular and metabolic impact of Bz-SSAO/VAP-1-dependent MET oxidation in generating H_2O_2 and formaldehyde has been extensively studied [5], less attention has been focused on Bz-SSAO/VAP-1 in modulating MET and NH_4^+ endogenous levels at different physio/pathological conditions characterized by altered concentrations of these basic compounds [6,7].

As already postulated for NH_4^+, MET may alter exocytotic activity of excitable cells mainly through two mechanisms: intravesicular alkalinization [8] or a block of different potassium currents in pre- or post-synaptic membranes [9]. From both these mechanisms, changes in animal behavior and/or neurological functions can be observed as a consequence of neurotransmitter release [10]. For NH_4^+, the ability to induce a blockage of voltage-operated potassium channels, thus producing the release of γ-aminobutyrric acid (GABA), glutamate, taurine, and nitric oxide (NO), has already been demonstrated, as well as changes in animal behavior [11,12].

Little indirect information is available on MET tissue and plasma levels and on its physiological role(s) in rodents and humans [13,14]. It is known that MET plasma and urinary levels increase following Bz-SSAO/VAP-1 inhibition [6] or in some pathological conditions such as diabetes, typhoid fever, and liver or renal insufficiency [15]. In parallel, in the plasma of diabetic patients [16] as well as in the plasma of animal models of type I or type II diabetes [17], Bz-SSAO/VAP-1 levels also increased, a finding that has aroused interest in the role of the enzyme and its substrate in diabetes. In this respect, recent data confirm that Bz-SSAO/VAP-1 catalysis generates and sustains the diabetes vascular complications, a finding in contrast to the opposite (beneficial) insulin-like effects of the H_2O_2 produced by MET degradation [18,19]. This latter finding makes the relationship between Bz-SSAO/VAP-1 activity and substrate concentration quite puzzling and underlines the need to explore MET (and NH_4^+) physiological role(s) in rodents and humans. All together, these results strongly support the view that the physiological significance of Bz-SSAO/VAP-1 can be better elucidated by improving our knowledge of the physiological role of its substrates and reaction end-products. The role of tissue and plasma MET levels in modulating central neurotransmitter release (as NH_4^+ does) has not yet been clarified.

THE ROLE OF METHYLAMINE IN THE CENTRAL NERVOUS SYSTEM

Recent data have shown that MET is able to interfere with the hypothalamic system involved in regulating food intake of fasted mice [20]. Feeding is a complex behavioral function in mammals, controlled by a network of hormones, peptides, and neurotransmitters, with anorexic and orexic signaling features whose reciprocal concentrations fluctuate at the hypothalamus. During fasting, this physiological clock shifts towards increasing levels of orexic mediators [21]. In fasted animals, MET, delivered intraperitoneally (i.p.) or directly into the central nervous system (i.c.v.), reduced, dose-dependently, food intake of healthy Swiss mice (i.p. EC50 = 334.6 mg/kg) without altering their locomotor and exploring activity. MET potency was improved when the amine was administered at the time of Bz-SSAO/VAP-1 inhibition [20].

a) What is the mediator involved in MET hypophagia?

Pharmacological evidence obtained in mice pre-treated with monoamine oxidase activity (MAO A or MAO B) inhibitors [20,22], dopaminergic or serotoninergic antagonists, and from microdialysis experiments in freely moving rats (unpublished results) confirm that MET did not evoke dopamine or serotonin release (both amines are MAO substrates), thus excluding the possibility that MET might have amphetamine-like effects. Hence, one plausible candidate responsible for MET hypophagia might have a peptidergic or aminoacidergic moiety.

b) Methylamine hypophagia depends on neuronal potassium channels

It is well known that neuron firing can be modulated at different levels. One way of controlling neurotransmitter release is to modify the gating activity of ion channels, among which are the potassium channels. MET hypophagia was reduced when the expression of the isoform Kv1.6 of the "Shaker-like" family (Kv1-7) was down-regulated by using a selective antisense oligodeoxyribonucleotide (aODN6). Interestingly, the reduced expression of the Kv1.6 reduced methylamine-dependent, but not ammonia-dependent hypophagia (Figure 1). This result suggests that, despite the large similarities, ammonia and methylamine interact with different neuronal potassium channels. At present, lacking direct electrophysiological data, we hypothesize that MET might induce hypophagia by controlling the neuronal release of some neurotransmitter(s), similarly to that which was already described for known blockers of neuronal voltage-operated potassium channels [23].

c) Is methylamine hypophagia maintained in hyperphagic, obese animals over-expressing Bz-SSAO/VAP-1?

Obesity is considered an important risk factor for cardiovascular diseases and for the onset of the metabolic syndrome. Both are consequences of bad life-style habits, typical of people from industrialized countries. Obesity is the result of

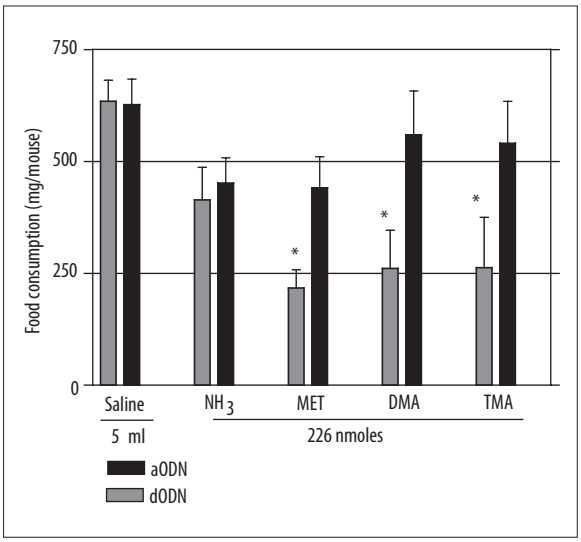

Figure 1. The hypophagic effects of methylamine, dimethyl, and trimethyl amine are selectively abolished by knocking down neuronal Kv.1.6 expression. Mice were injected i.c.v. with dODN and aODN (9 nmol per single injection on days 1, 4, and 7). Mice were starved overnight and drugs were injected i.c.v. (5 ml) under light anesthesia. Food consumed by each mice was evaluated for 60 min after drug administration.

an imbalance between energy intake and its expenditure. This complex ratio is regulated by a plethora of peripheral or central signals, such as leptin, which modulate the firing of hypothalamic neurons, especially those located at the ventromedial hypothalamus and the arcuate nucleus [24]. Leptin is an adipocyte hormone which signals at the hypothalamus to stop feeding and is a key component of the long-term system which maintains body weight. Through the activation of receptors, it negatively controls the release of NPY/agouti (two strong orexigen peptides), whereas it increases the release of other anorexic mediators.

Obesity can be reproduced in animals in different ways, including genetic manipulation. Rodents in which either the gene coding for leptin (ob/ob mice) or for its hypothalamic receptor (db/db mice and fa/fa rats) have been silenced are obese (animals with overstimulation of feeding and reduced energy expenditure) and soon develop all the neurological and behavioral signs of the insulin-resistance syndrome. Obese mice represent an important experimental model to verify whether MET hypophagia is maintained when the expression of its metabolizing activity (from adipocytes and plasma) is high.

Methylamine Hypophagia in Obese Mice

The hypophagic effect of MET was investigated in 4 month old C57Jdb/db (db/db) obese (and type II diabetic) mice and in their counterpart, lean animals (db/db$^+$) bearing the heterozygous deletion of the leptin receptor. The characteristics of the mice are shown in Table 1. Exogenous MET injected i.c.v. (30 and 75 µg), or i. p. (from 50 to 400 mg/kg) produced a similar dose-dependent hypophagia in both obese and fasted lean mice, despite the fact that obese mice had higher levels of the Bz-SSAO/VAP-1 activity than the lean mice. Interestingly, in the adipose tissue of obese animals, the mRNA levels for Bz-SSAO/VAP-1 were lower than those measured in db/db$^+$ (data not shown). This finding is an agreement with previous observations obtained in Zucker rats and supports the hypothesis that Bz-SSAO/VAP-1 activity may directly or indirectly control its mRNA levels (27). In contrast, no significant differences were observed between db/db and db/db$^+$ animals in the mRNA levels for brain Kv1.6 channels. Notably, brain Bz-SSAO/VAP-1 activity in db/db and db/db$^+$ animals was clearly measurable.

Table 1. Some metabolic characteristics of 4 month old db/db and db/db$^+$ mice.

	db/db$^+$	db/db
Body weight (g)	30.7±0.6	53.1±2.9
Epidydimal fat (g) (% of body eight)	1.2±0.2	4.8±0.7
Plasma glycemia (mM)	10	>20
Adipose tissue Bz-SSAO activity (nmoles/g/15 min)	11.2±0.1	25.5±0.7*

Animals were fasted overnight and sacrificed. Plasma glycemia was evaluated spectrophotometrically (Sigma-Aldrich). Bz-SSAO activity of white adipose tissue was evaluated radiochemically using ^{14}C-benzylamine (50 µM) as substrate. (*Significantly different from the db/db$^+$, $p<0.05$).

a) The role of Bz-SSAO/VAP-1 in obese mice

The role of Bz-SSAO/VAP-1 in both animal strains in controlling MET central levels was estimated using animals pre-treated with α-amino guanidine (α-AMG; 50 mg/kg i.p.). Under these experimental settings, an increase in MET (i.p) hypophagic potency was observed only in the obese and not in the lean ones (EC50 was shifted significantly to the left), whereas in both animal strains the α-AMG treatment produced a significant potentiation of MET hypophagia when it was injected i. c. v. These results allow the conclusions that 1) C57BL animals were more sensitive to MET hypophagia than Swiss albino mice, 2) the increase in MET potency in db/db mice after α-AMG, mirrors the higher availability of the Bz-SSAO/VAP-1 in obese animals, 3) brain Bz-SSAO/VAP-1 has a significant role in controlling MET central levels in both animal strains, 4) the leptin circuit is not involved in MET hypophagia, and 5) the effect of α-AMG on the potentiation of MET hypophagia might be an aspect of the beneficial effects reported for the drug in diabetes. Moreover, since MET and Bz-SSAO/VAP-1 levels increased in diabetes, this high MET metabolizing activity might account, at least partially, for the hyperphagia which characterize the diabetic animals.

Methylamine and Nitric Oxide: A Further Interplay between the Amine and Potassium Channels

Recent experiments show that MET increases NO levels in the hypothalamus. In fasted rats, at doses that do not affect food consumption, MET (5 and 15 µg injected i.c.v.) produces a time-dependent increase in NO (from 0.2 to 1 µM in microdialysate samples from hypothalamus. This effect

was antagonized by L-NAME (2 µmol, i.c.v.) and it was also significantly reduced (–52.7%) in animals bearing the expression of potassium channel (Kv1.6) knocked down by pretreatment with a selective aODN. These preliminary results provide the first evidence that MET, by interacting with Kv1.6 channels, induces the release of non-monoaminergic mediators in the brain. Moreover, because an NO-releasing activity was also described as a sequential mechanism during acute ammonia neurotoxicity (28), the present data support the view that MET could take place, with NH_4^+, in central neurodegenerative pathologies.

Methylamine and Other Alkyl-Amines

In addition to NH_4^+ and MET, dimethyl and trimethylamine (DMA and TMA) and trimethyl amine (TMA) also occur endogenously at low concentrations as the end-products of nitrogen metabolism. Interestingly, DMA is produced by the degradation of nitro arginine derivatives, among which is the asymmetric dimethyl arginine (ADMA), the most potent endogenous inhibitor of NO synthase activity. ADMA is degraded by a dimethyl arginine dimethyl amino hydrolase activity (EC 3.5.3.18), producing citrulline and DMA.

We have verified that DMA and TMA still maintain the hypophagic effect of MET and NH_4^+ described in healthy fasting mice. As it occurred for MET, DMA and TMA hypophagia was still dependent on Kv1.6 expression, whereas NH_4^+ hypophagia was not (Figure 1). Unlike MET, TMA, and DMA are not related to Bz-SSAO/VAP-1 activity. Nothing is known yet about the ability of TMA and DMA to evoke NO release. If it were be found, a link between plasma and tissue levels of NO synthase inhibitors (nitro arginines, ADMA) and of NO producers (short amines) might be postulated. This might represent an additional pathway in controlling NO availability, explaining vascular complications observed in several pathologies characterized by nitrogen metabolic derangements [27].

Conclusions

MET and NH_4^+, two short amines which are, respectively, a substrate and an end-product of Bz-SSAO/VAP-1 activity, have their own features in the central nervous system. The two amines, targeting at different neuronal voltage-operated potassium channels, induce both reduction of food intake and also the release of NO [28], a finding which opens new perspectives in the study of the physio/pathological role of MET, with particular regard to neurodegenerative pathologies where short-amine central levels increase. Pharmacological modulation of MET, and then of ammonia central levels, can be achieved by using α-AMG, a Bz-SSAO/VAP-1 inhibitor, a strategy that could play an important role in controlling food intake and weight gain in obese animals without inducing amphetamine-like effects.

The substitution of an ammonia hydrogen(s) with a methyl group(s) (from MET to DMA and TMA) appears to be a condition for distinguishing the type of neuronal Kv1.6 channels blocked. Despite this chemical selectivity, MET, DMA TMA, and NH_4^+ are all hypophagic, and MET and NH_4^+ release NO at hypothalamic nuclei.

The evidence suggests that 1) Bz-SSAO/VAP-1 activity is essential in controlling hypophagic mediator levels in the central nervous system, 2) there is a link between the NO pathway and Bz-SSAO/VAP-1 activity, and 3) small alkyl derivatives of NH_4^+ might be included among other important known endogenous modulators of tissue and cell functions.

The monitoring of the tissue levels of these short amines is now the first-line aim of future investigations in this field.

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