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Short communication

Pharmacological antagonism of lipoprivic feeding induced by sodium mercaptoacetate

Vittorio L. Garosi [a], Enzo Nisoli [a,*], John E. Blundell [b], Michele O. Carruba [a]

[a] *Section of Pharmacology, Toxicology, and Experimental Therapeutics, School of Medicine, University of Brescia, Via Valsabbina 19, 25123 Brescia, Italy*

[b] *BioPsychology Group, Psychology Department, University of Leeds, Leeds, LS2 9JT, UK*

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Abstract

Drugs, such as sodium mercaptoacetate and methylpalmoxirate, which block fatty acid oxidation at different levels in the metabolic pathway, stimulate feeding. It is well known that selective centrally induced stimulation of dopamine, serotonin (5-hydroxytryptamine, 5-HT) and β-adrenoceptors, or inhibition of the opiatergic system substantially decrease food intake in rats trained to eat 4 h a day. The results of the present study show that centrally acting dopaminergic and serotoninergic anorectic drugs, the opiate receptor antagonist naloxone, the α-adrenoceptor blocking drug phentolamine, and peripherally administered 5-HT counteract the overeating induced by mercaptoacetate. Comparing these effects to those described in 2-deoxy-D-glucose- and insulin-induced feeding, our data support the proposition that distinct neural circuits are involved in the hyperphagic responses to diverse metabolic stimuli.

Keywords: Hyperphagia, experimentally induced; Anorectic drug; 2-Deoxy-D-glucose; Insulin; Sodium mercaptoacetate; Neural circuit

1. Introduction

It is a widely accepted notion that feeding ultimately must subserve both the unique metabolic requirements of brain and body weight homeostasis. Therefore, it seems reasonable to expect that some important controls of food intake would arise from metabolic cues. Until recently, cerebral glucoprivation, induced by glucose antimetabolites, such as 2-deoxy-D-glucose or 5-thio-D-glucose, or by hypoglycaemic doses of insulin (Ritter and Slusser, 1980), was the most studied experimentally induced hyperphagia (Carruba et al., 1985). More recently it was shown that hepatic oxidation of metabolic fuels may provide a post-absorptive satiety signal and hence it may be involved in controlling feeding (Langhans and Scharrer, 1987a,b). In this line, it was found that rats increase their food intake in response to blockade of fatty acid oxidation (i.e., lipoprivation). Indeed, drugs, such as sodium mercaptoacetate and methylpalmoxirate, which block fatty acid oxidation at different levels in the metabolic pathway, stimulate feeding (Scharrer and Langhans, 1986). They are mostly active in rats made dependent on fat metabolism by adaptation to a fat-supplemented diet (Scharrer and Langhans, 1986). The various stimuli arising from the blockade of glucose and fat metabolism appear to be centrally integrated in controlling food intake (Friedman and Tordoff, 1986).

At the present time it appears that there are anatomical distinctions in the pathways responsive to different metabolic challenges arising from the blockade of fat and glucose metabolism (Ritter and Taylor, 1989). In previous experiments we have examined the effect of various types of drugs on the hyperphagia induced by 2-deoxy-D-glucose and insulin (Carruba et al., 1985). This strategy has provided some insights into the pathways mediating this metabolically induced hyperphagia. In the present study we have examined the

* Corresponding author. Sezione di Farmacologia, Tossicologia e Terapia Sperimentale, Dipartimento di Scienze Biomediche e Biotecnologie, Università degli Studi, Via Valsabbina, 19, 25123-Brescia, Italy. Tel. 0039 (30) 3715290 – 3715292, fax 0039 (30) 3701157.

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effects of a range of anorectic drugs on the hyperphagia induced by the inhibition of fat oxidation. This approach will not identify the pathways responsible for this hyperphagia arising from fat oxidation, but it should disclose which neurotransmitter systems are involved (or not) in mediating the phenomenon.

2. Materials and methods

2.1. Animals and housing conditions

Adult male Sprague-Dawley rats (Charles River, Italy), 275–300 g body weight, were used in this study. They were housed in groups of three in Makrolon cages upon arrival in a temperature-controlled room (21 ± 0.5° C) on a 12:12 h light-dark cycle (lights on 07:00 a.m.). All behavioural testing was done in the rats' home cages. Food (see below for special diet) and tap water were available ad libitum at all times.

A group of rats was trained to eat a medium-fat powdered diet (18% fat; Laboratori Piccioni, Italy. Percentage of the various components of the diet: pure milk casein: 13; corn starch: 48; soya oil: 3.4; lard: 14.6; Wesson mineral mixture: 4; vitamin mixture: 1; pure cellulose: 16). The other group ate a pelleted lab chow. The medium-fat, high carbohydrate diet used in our studies was adequate for expression of a feeding response to mercaptoacetate-induced blockade of fat metabolism. Feeding tests were conducted beginning 2 h after the start of the light period.

2.2. Feeding tests

After 15 days the rats adapted to diet and maintenance conditions were placed in individual cages, and 24 h later they were injected i.p. with dexfenfluramine, *m*-chlorophenylpiperazine, quipazine, 5-HT, *d*-amphetamine, lisuride, salbutamol, phentolamine, and naloxone. These drugs were injected at different doses, including those causing inhibition of feeding by 50% (ID_{50}) in rats trained to eat 4 h a day (Carruba et al., 1985). The rats were then injected with sodium mercaptoacetate (400 μmol/kg i.p.), to block fatty acid oxidation, or saline. Intake of the maintenance diet was measured hourly for 6 h beginning immediately after injection of drug or saline.

The dose of mercaptoacetate was chosen as that reported to cause a constant and substantial increase in food consumption (Scharrer and Langhans, 1986).

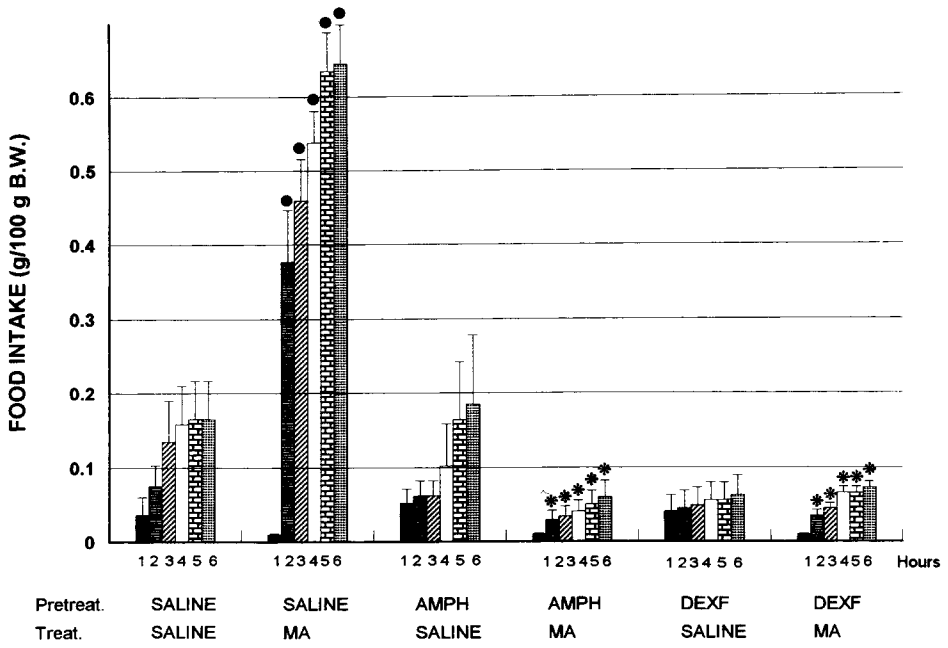

Fig. 1. Effects of *d*-amphetamine (AMPH) and dexfenfluramine (DEXF) on mercaptoacetate (MA)-induced hyperphagia. The ID_{50}s of *d*-amphetamine and dexfenfluramine were given i.p. to rats, trained to eat a medium-fat diet, before mercaptoacetate (400 μmol/kg i.p.) or saline injection, and the food intake was measured hourly for 6 h. The weight of food consumed was converted to g per 100 g of body weight. Data are representative of a single experiment with six experimental groups performed in the same experimental session. Six rats per group were used. Bars represent means ± S.E. of cumulative amounts of food eaten hourly within 6 h after saline or mercaptoacetate administration, and data were statistically analized by analysis of variance together with the Newman-Keuls' multiple comparisons post-hoc test. The overall between-times significance was not statistically significant ($F = 0.26$), and the significance of the 2-factor interactions (treatment × pretreatment) was < 0.01 and, according to the Newman-Keuls' multiple comparisons test, this was due to saline + mercaptoacetate treatment. $^{\bullet}$ $P < 0.01$ vs. saline + saline; * $P < 0.01$ vs. saline + mercaptoacetate.

2.3. Statistical analysis

The weight of food consumed was converted to g per 100 g of body weight. Data are presented as means ± S.E. and were statistically analysed by analysis of variance together with Newman-Keuls' multiple comparison post-hoc test.

2.4. Materials

The following drugs were used: sodium mercaptoacetate (Sigma, Milan, Italy), *d*-amphetamine sulphate (Recordati, Milan, Italy), salbutamol sulphate (Valeas, Milan, Italy), lisuride hydrogen maleate (Schering, Berlin, Germany), dexfenfluramine hydrochloride (Laboratoires Servier, Orleans, France), quipazine hydrogen maleate (Miles Laboratoires, Elkhart, USA), 5-hydroxytryptamine hydrochloride (Sigma, Milan, Italy), *m*-chlorophenylpiperazine dihydrochloride (RBI, Amity, Milan, Italy), phentolamine hydrochloride (Ciba-Geigy, Basel, Switzerland), and naloxone hydrochloride (ENDO Labs, Garden City, NY, USA). Drugs were dissolved in saline and administered i.p. in a volume of 0.5 ml per 100 g of body weight. Anorectic drugs were given 15 min before mercaptoacetate, except *d*-amphetamine and dexfenfluramine, which were given 30 min before, and phentolamine, which was given immediately before. Injection times were selected so that each drug was tested at the time of its peak action.

3. Results

As previously shown (Scharrer and Langhans, 1986), mercaptoacetate (400 μmol/kg i.p.) stimulated feeding in rats fed medium fat diet, without significantly influencing the food intake of the rats given low fat diet, when compared with controls. The stimulatory effect of mercaptoacetate was already present within 2 h after the administration, and this increment persisted until 6 h from the injection.

In order to evaluate their effects on mercaptoacetate-induced hyperphagia, various drugs, including some anorectic agents, were given i.p. to rats before mercaptoacetate or saline injection, and the food intake was measured for 6 h. Different doses of the drugs were tested to evaluate a dose-response relationship. In order to compare the effects on mercaptoacetate to those on other hyperphagic models we report on the effects of drug doses corresponding to that causing 50% inhibition of food intake (ID_{50}) in rats trained to eat 4 h a day (Carruba et al., 1985).

Fig. 1 shows that the i.p. injection of the ID_{50}s of *d*-amphetamine (5.43 μmol/kg) or dexfenfluramine (5.49 μmol/kg) counteracted the increase in food intake induced by mercaptoacetate. The inhibitory effects of the ID_{50}s of several other anorectic drugs, that act upon either the dopaminergic (lisuride 0.12 μmol/kg), or serotoninergic (*m*-chlorophenylpiperazine 43.3 μmol/kg, quipazine 7.23 μmol/kg) systems on mercaptoacetate-induced overeating were studied. Dopaminomimetics or serotoninomimetics behaved like *d*-amphetamine or dexfenfluramine, respectively, in antagonising the hyperphagic effect induced by mercaptoacetate (Fig. 2). 5-HT itself, given systemically at a dose (13 μmol/kg) previously shown to reduce food intake in rats trained to eat 4 h a day (Carruba et al., 1986), also completely counteracted the hyperphagic response induced by mercaptoacetate (Fig. 2). The β_2-adrenoceptor agonist salbutamol reduced the mercaptoacetate-induced feeding (Fig. 2), when injected at a dose (26 μmol/kg) that was shown to induce a 50% reduction of food intake in rats trained to eat 4 h a day (Borsini et al., 1982; Garosi and Carruba, unpublished

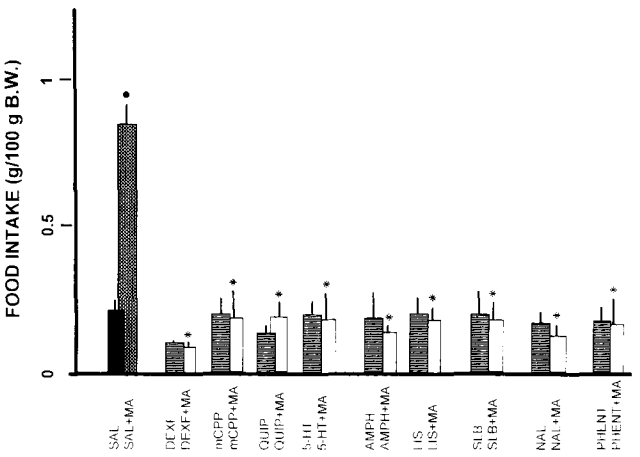

Fig. 2. Effects of various drugs on the mercaptoacetate-induced increase in food intake. Rats were given i.p.: saline + saline (black first column); saline + mercaptoacetate (MA, 400 μM/kg i.p.) (cross-hatched second column); dexfenfluramine (DEXF), or *m*-chlorophenylpiperazine (mCPP), or quipazine (QUIP), or 5-HT, or *d*-amphetamine (AMPH), or lisuride (LIS), or salbutamol (SAL), or naloxone (NAL), or phentolamine (PHENT) + saline (grey columns); the drugs to be tested + mercaptoacetate (MA, 400 μM/kg i.p.) (open columns). The various drugs were given at doses representing the ID_{50} concentrations in rats trained to eat 4 h a day. Naloxone was given at a dose of 10 mg/kg that causes an already maximal effect in respect to controls, and phentolamine was given at a dose of 1 mg/kg that does not change food intake in rats trained to eat 4 h a day. Bars represent mean values ± S.E. of cumulative amounts of food eaten within 6 h after saline or mercaptoacetate administration expressed as g per 100 g of body weight. At least 18 rats per group were used. Data refer to the mean of different experiments performed in different experimental sessions and were statistically analyzed by analysis of variance together with Newman-Keuls' multiple comparisons post-hoc test. The overall between-effects significance was not statistically significant ($F = 0.47$), and the significance of the 2-factor interactions (treatment × pretreatment) was < 0.01 and, according to the Newman-Keuls' multiple comparisons test, this was due to saline + mercaptoacetate treatment. $^{\bullet} P < 0.01$ vs. saline + saline; $^{*} P < 0.01$ vs. saline + mercaptoacetate.

observations). Naloxone, given at a dose of 10 mg/kg that causes an already maximal effect in respect to controls (40–45% reduction of food intake) in rats trained to eat 4 h a day, significantly counteracted the mercaptoacetate-induced hyperphagia (Fig. 2). All the drugs counteracted the mercaptoacetate-induced feeding in a dose-dependent manner and even when tested at dosages that per se only minimally change food intake (data not shown). Finally, Fig. 2 also shows that 1 mg/kg of the α_2-adrenoceptor antagonist phentolamine, that did not change food intake in rats trained to eat 4 h a day, was able to reduce the lipoprivation-induced feeding. The inhibitory effects on the mercaptoacetate-induced hyperphagia of all tested drugs persisted throughout 6 h of the experiment, except phentolamine, the effect of which persisted only for the first 4 h.

4. Discussion

The results of the present study have confirmed that mercaptoacetate-induced feeding is a reliable phenomenon which can be readily demonstrated when rats are offered a diet high in fat. However, this form of lipoprivic feeding is susceptible to antagonism by a wide range of pharmacological agents which inhibit eating induced by food deprivation. As shown in Fig. 2 all of the drugs used in this study, when administered prior to mercaptoacetate, prevented the increase in eating normally induced by mercaptoacetate. Moreover, it should be noted that the antagonism is not simply due to the summation of two opposing effects since care was taken also to ensure that the drugs used to antagonise mercaptoacetate-induced eating did not, at some of the doses used, lead to any marked inhibition of eating (data not reported). In addition, phentolamine which per se does not modify feeding, was effective in antagonising lipoprivic feeding. Therefore the effects observed do appear to be due to an antagonism via pharmacologically specific receptors or pathways.

How should these results be interpreted? One interpretation is that the neural circuitry involved in lipoprivic feeding includes all of the neurotransmitters (at either peripheral or central sites) modulated by the pharmacological agents administered. This means that there is little or no pharmacological specificity mediating the metabolic and behavioural responses to mercaptoacetate administration. This would not be surprising in view of the complexity of the neural system subserving the expression of eating (see for example Blundell, 1991).

Another interpretation is that mercaptoacetate-induced feeding, although a reliable phenomenon, could be extremely sensitive to any physiological adjustment (brought about by drugs) which occurs under these experimental circumstances. At the present time there does not appear to be any pharmacological challenge which fails to prevent mercaptoacetate-induced feeding. However, given the robustness of the induction of feeding by mercaptoacetate it would be illogical to conclude that it is both robust and fragile.

Perhaps the most relevant interpretation of these data is to compare them with the effects of drugs on feeding induced by 2-deoxy-D-glucose and by insulin. Our data support the proposition that different circuits are involved in the hyperphagic responses to different metabolic stimuli (Carruba et al., 1985). In line, it was shown that mercaptoacetate- but not 2-deoxy-D-glucose-induced feeding requires vagal sensory neurons that terminate in the area postrema/nucleus of the solitary tract (Ritter and Taylor, 1989). Furthermore, our previous work showed that different neuronal or humoral circuits underlie 2-deoxy-D-glucose- and insulin-induced eating (Carruba et al., 1985). While 2-deoxy-D-glucose needed functioning α_2-adrenoceptors to bring about increased food intake, insulin does not (Carruba et al., 1986), and serotoninergic and dopaminergic systems modulate the eating responses of rats to cerebral glucoprivation induced by 2-deoxy-D-glucose or by insulin differently (Carruba et al., 1985). Dexfenfluramine, *d,l*-fenfluramine, *p*-chloroamphetamine, fluoxetine, and quipazine, which cause anorexia by enhancing brain serotoninergic neurotransmission through different mechanisms, were shown to antagonise both insulin- and 2-deoxy-D-glucose-induced hyperphagia, whereas *d*-amphetamine, diethylpropion, lisuride, and bromocriptine, which induce anorexia primarily by enhancing brain dopaminergic neurotransmission (Carruba et al., 1980), were only effective in antagonising the hyperphagia induced by 2-deoxy-D-glucose but not that induced by insulin (Carruba et al., 1985). In addition to central serotoninergic pathways, a peripheral 5-HT mechanism, activated by systemic administration of 5-HT, has been shown to reduce food intake in rats trained to eat 4 h a day and to counteract both insulin and 2-deoxy-D-glucose hyperphagias (Carruba et al., 1986). This peripheral 5-HT mechanism seems to be relevant also in the lipoprivation-induced hyperphagia. Indeed, peripherally administered 5-HT, which does not cross the blood-brain barrier, completely antagonised the increase in food intake induced by mercaptoacetate. The neurochemical mediators of 2-deoxy-D-glucose-, insulin-, and mercaptoacetate-induced feeding are not all completely known. Nevertheless, Koegler and Ritter (1993) found that galanine terminals, possibly in the area postrema/nucleous of the solitary tract, may mediate mercaptoacetate- but not 2-deoxy-D-glucose-induced feeding, providing additional evidence of involvement of distinct neural circuits. In this line, the present work shows that the

α_2-adrenoceptor antagonist, phentolamine, antagonises the mercaptoacetate-induced hyperphagia, indicating the need for functionally active α_2-adrenoceptors in lipoprivation-induced feeding, similar to 2-deoxy-D-glucose-induced hyperphagia but unlike insulin-induced overeating (Carruba et al., 1985). The opiate antagonist naloxone, which is able to reduce mercaptoacetate-induced feeding, had been previously shown to counteract the 2-deoxy-D-glucose-, but not the insulin-induced hyperphagia (Carruba et al., 1985). Finally, we have recently observed that the β_2-adrenoceptor agonist, salbutamol, counteracted the insulin- and mercaptoacetate-induced hyperphagias, but not that induced by 2-deoxy-D-glucose (Garosi et al., submitted).

In this context it is also noteworthy that the activation of either central or peripheral serotoninergic systems is able to reduce both glucoprivation- and lipoprivation-induced hyperphagias. In addition, the peculiar modulatory effects of serotoninergic drugs, such as dexfenfluramine, on dietary fat intake (Blundell and Lawton, 1993; Lafreniere et al., 1993) and on the peripheral lipid metabolism, should be taken into account when interpreting the complex networks that control feeding induced by blockade of fat metabolism. It is worth recognising that the activation of 5-HT receptors will apparently antagonise every form of experimentally induced feeding, including eating stimulated by metabolic adjustment. This could mean that 5-HT pathways are involved in one neural component common to all types of feeding such as a final output pathway for the expression of eating, or for some integration of metabolic need with behavioural activity. The type of study conducted here provides an additional piece of evidence to help understand the very complex systems which subserve feeding in response to true biological needs of animals.

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