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The acute effect of amylin and salmon calcitonin on energy expenditure

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Abstract

The pancreatic B-cell hormone amylin is known to be involved in the regulation of meal ending satiation and it also shares typical features of adiposity signals. Chronic amylin administration has recently been shown to increase energy expenditure under certain conditions. Here we investigate the acute effect of peripheral administration of amylin or its agonist salmon calcitonin (sCT) on energy expenditure and respiratory quotient (RQ). First, rats were injected with amylin (5 μg/kg IP) or saline just before dark onset. Despite significantly decreased food intake in amylin-treated rats compared to control until 2 h post-injection ($p<0.05$), amylin did not influence energy expenditure or RQ. Reduced food intake, which reduces energy expenditure, may have confounded a stimulatory effect of amylin on energy expenditure. Therefore, in the second experiment, amylin (1, 5 and 10 μg/kg IP) or saline was injected in the middle of the light phase ($t=0$ h) without access to food during 3 h post-injection. Amylin had no significant effects on energy expenditure or RQ. In a similar paradigm, the effect of sCT (0.1, 1.0 and 5.0 μg/kg IP) was tested. During food restriction, 5.0 μg/kg sCT significantly stimulated energy expenditure compared to control ($p<0.05$). Subsequent to refeeding at $t=3$ h, energy expenditure was decreased compared to control at $t=8$ h and $t=10$ h after 5.0 μg/kg sCT, probably due to sCT's strong anorectic action. Thus amylin may prevent the compensatory decrease in energy expenditure normally seen in animals that eat less. The longer acting sCT stimulated energy expenditure in animals without food access.

Keywords: Amylin; Salmon calcitonin; Energy expenditure; RQ; Physical activity; Body temperature; Food intake

1. Introduction

Amylin is a 37 amino acid peptide hormone which is co-secreted with insulin by the pancreatic B-cell in response to food intake [1]. Many studies have shown that amylin potently reduces food intake after both peripheral and central administrations [2–8]. Acute peripheral delivery of amylin has been shown to inhibit food intake mainly by reducing meal size [6]. Amylin's anorectic effect is not due to the induction of conditioned taste aversion [6,8–10].

Besides the function as a short-term satiation signal, amylin also has a long-term anorectic and body weight reducing effect. Chronic administration of amylin reduces food intake and body weight in rats [11,12]. This may implicate that amylin acts as an adiposity signal [13], similar to leptin and insulin [14]. Furthermore, amylin deficient knockout mice show an increased body weight gain compared to wild type control mice [15,16]. Interestingly, the amylin knockout mice did not show a difference in food intake (unpublished). The latter could indicate that energy expenditure might be decreased in these knockout mice.

In addition to the indirect evidence from the amylin knockout mice, other data indicate that amylin may influence energy expenditure. Two early publications suggest that central amylin increases body temperature [2,17], although a high dose was used. Further, rats which were food deprived for 2 days lost more weight when treated daily with amylin's agonist salmon calcitonin (sCT) [18] than fasted control rats [13]. Because in this experiment all rats did not have access to food, these data may also suggest that sCT stimulates energy expenditure and thereby reduces body weight more than in the controls [13]. Finally, a recent publication showed that under certain circumstances chronic infusion of amylin by osmotic minipumps increased energy expenditure in rats [19].

Collectively, these data may suggest that amylin or its agonist sCT stimulates energy expenditure. Therefore, the aim

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of the present studies was to investigate whether amylin or sCT stimulates energy expenditure when administered acutely.

2. Materials and methods

2.1. Animals and housing

Ten male Wistar rats (Elevage Janvier, Le-Genest-St. Isle, France) weighing 300–330 g at the beginning of the study were individually housed in Plexiglas air tight metabolic cages (41×41×31 cm) on a layer of wood shavings under artificial 12 h/12 h light–dark cycle (lights on 03:00 h to 15:00 h) and at a room temperature of 21±2 °C. Water and standard powder chow (GLP 3433, Provimi Kliba AG, Kaiseraugst, Switzerland) were available ad libitum, unless otherwise stated. The rats were adapted to the housing conditions for at least 2 weeks before the tests. All experiments were approved by the Veterinary Office of the Canton Zürich, Switzerland.

2.2. Surgery

Under brief isoflurane anesthesia, all rats were implanted IP with a temperature transmitter (VM-FA disc, MiniMitter, Bend OR).

2.3. Indirect calorimetry

Measurements were conducted in an open circuit calorimetry system (AccuScan Inc., Columbus, OH). Room air was passed through each cage with a flow rate controlled at approximately 2 l/min. Every 2 min, out-coming air was sampled during 20 s for each individual cage and analyzed for O_2 and CO_2. Simultaneously, physical activity was monitored by 3 arrays of 16 infrared light beam sensors. Furthermore, food intake and water intake were measured continuously. All data were analyzed with AccuScan Integra ME software. Energy expenditure was calculated for each 2 min sample according to Weir [20] using the following equation: total energy expenditure (kcal/h)=3.9×V(O_2) L/h+1.1×V(CO_2) L/h. The average over 30 min or 60 min was calculated for each individual animal and expressed as kcal/h. The respiratory quotient (RQ) was defined as the quotient of CO_2 production and O_2 consumption. The light beam breaks were converted into distance traveled in cm.

2.4. Experimental design

In the first experiment, the effect of amylin on energy expenditure was measured in a randomized cross-over design with at least 2 days between trials. Twenty minutes before dark onset, the cages were briefly opened and the rats were injected IP with 5 μg/kg amylin (Bachem AG, Bubendorf, Switzerland; dissolved in 1 ml saline) or the same volume of saline as control. The rats were returned to the cages which were closed immediately. Ten minutes later, i.e. at dark onset, the measurement was started. All parameters were measured during the subsequent 24 h.

The second experiment was performed in the middle of the light phase. In this experiment, the access to the food hopper was blocked from 30 min before to 3 h after injection. Twenty minutes before the middle of the light phase, the cages were briefly opened and amylin (1.0, 5.0 or 10.0 μg/kg) or saline as control was injected IP. The rats were returned to the cages which were closed immediately. Ten minutes later the measurement was started. Access to food was given 3 h after injection. All parameters were measured during 24 h, i.e. for 3 h without the rats having access to food and for the subsequent 21 h when the rats could feed freely.

In the third experiment with an identical design as experiment 2, the effect of amylin's agonist salmon calcitonin (0.1, 1.0 or 5.0 μg/kg IP, Bachem AG) or saline as control on energy expenditure was measured. Because sCT is longer acting than amylin [18], in this experiment the trials were separated by at least 3 days.

2.5. Statistical analysis

The data are expressed as mean±SE over 30 min or 60 min. One-way ANOVA with repeated measures and post-hoc Bonferroni test for each time point were used to test for significant differences. $p<0.05$ was considered significant.

3. Results

In rats with ad libitum access to food, amylin (5 μg/kg) had no effect on energy expenditure compared to controls when injected just before dark onset (Fig. 1A). This dose significantly reduced food intake compared to controls at 30 min and 2 h after injection ($p<0.05$, Fig. 1B). Water intake was decreased to a similar degree as food intake ($p<0.05$, Fig. 1C). Energy expenditure, RQ, physical activity and body temperature were not significantly different from control (data not shown).

In the second experiment, amylin was injected in the middle of the light phase at 3 different doses in rats that had no access to food for 3 h after injection. As expected in animals without access to food, energy expenditure significantly decreased over time in all groups ($F_{(3,108)}=19.45$, $p<0.001$). Amylin appeared to slightly, but non-significantly increase energy expenditure for the first 30 min into the study, but overall no significant effect of amylin on energy expenditure was observed during the 3 h without access to food (Fig. 2). RQ, physical activity and body temperature after amylin treatment were not significantly different from control (data not shown). When food was returned at $t=3$ h after injection, no significant difference in any of the measured parameters was observed (data not shown).

Fig. 3 shows the effect of different doses of sCT on energy expenditure during the first 3 h of the experiment when the rats had no access to food. Energy expenditure significantly decreased over time ($F_{(3,108)}=7.98$, $p<0.001$). ANOVA revealed a significant effect of treatment at $t=120$ min ($F_{3,9}=6.59$, $p=0.002$) and $t=180$ min ($F_{3,9}=7.54$, $p<0.001$). Post-hoc analysis showed that energy expenditure was significantly increased in rats injected with sCT (5.0 μg/kg) at $t=120$ ($p<0.001$) and at $t=180$ min ($p<0.001$) compared to control. The 1.0 μg/kg dose just failed to reach significance compared to control at $t=120$ min ($p=0.07$). RQ, physical activity, body temperature