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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

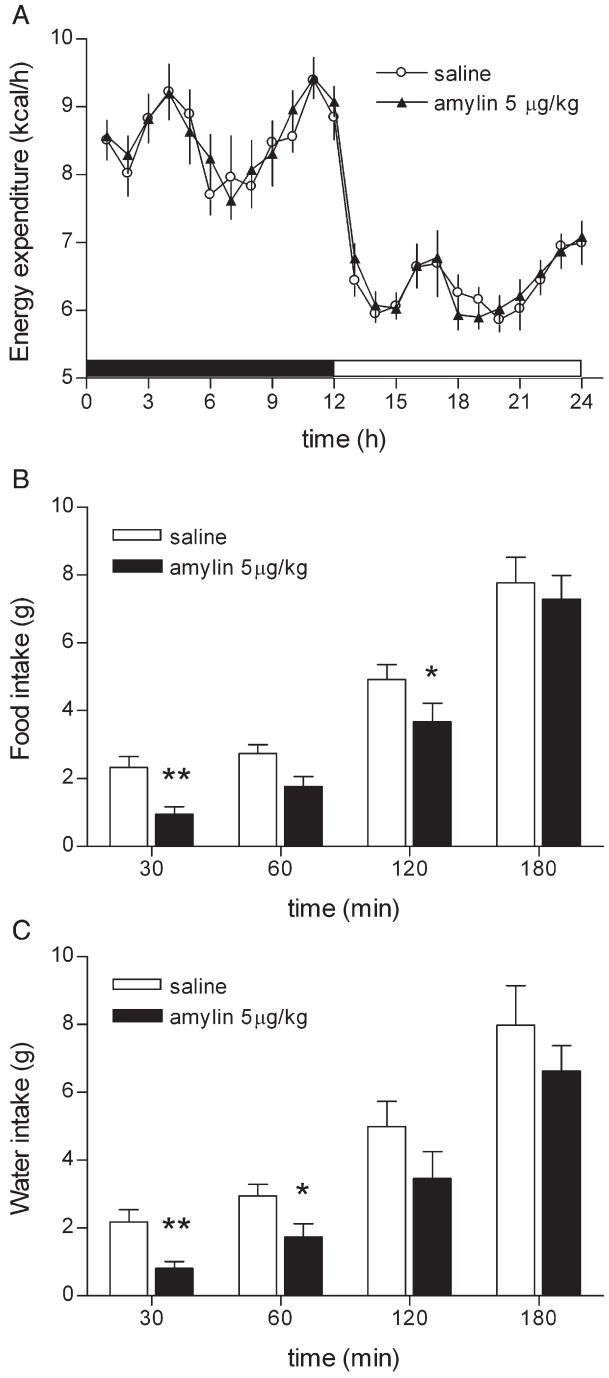

Fig. 1. A: Effect of amylin (5 μg/kg IP) or saline, on energy expenditure. Rats ($n=10$) were injected in a randomized cross-over design 10–20 min before dark onset. Data are expressed as mean±SE for every hour. B: Effect of amylin (5 μg/kg IP) or saline, on cumulative food intake over the first 3 h after injection. Rats ($n=10$) were injected in a randomized cross-over design 10–20 min before dark onset. Data are expressed as mean±SE. *$p<0.05$, **$p<0.01$. C: Effect of amylin (5 μg/kg IP) or saline, on cumulative water intake over the first 3 h after injection. Rats ($n=10$) were injected in a randomized cross-over design 10–20 min before dark onset. Data are expressed as mean±SE. *$p<0.05$, **$p<0.01$.

and water intake were not significantly different between the groups at any time point during the food restriction (data not shown). Fig. 4A shows the time course of energy expenditure

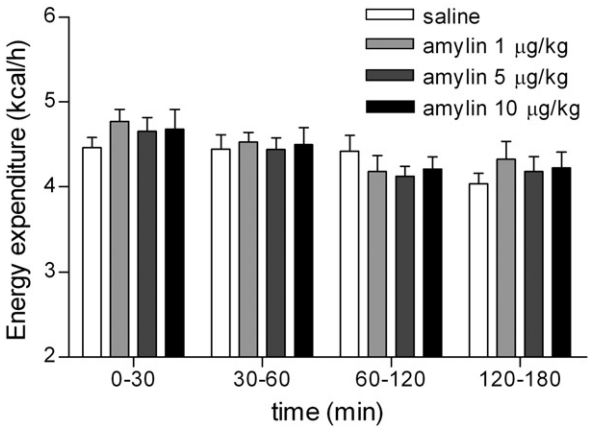

Fig. 2. Effect of amylin (1 μg/kg, 5 μg/kg or 10 μg/kg IP) or saline on energy expenditure. Rats ($n=10$) were injected in a randomized cross-over design in the middle of the light phase without access to food for 3 h after the injection. Data are expressed as mean±SE per time interval, normalized to kcal/h.

over the complete 24 h experimental period. Energy expenditure was significantly different between groups at $t=7$ h ($F_{3,9}=4.93$, $p<0.01$), $t=8$ h ($F_{3,9}<4.02$, $p=0.017$) and $t=10$ h ($F_{3,9}=7.688$, $p<0.001$), i.e. starting from 4 h after refeeding. Post-hoc analysis showed significantly decreased energy expenditure in rats injected with 1.0 μg/kg sCT compared to controls ($p=0.016$) at $t=7$ h and in rats injected with 5.0 μg/kg at $t=8$ h ($p=0.026$) and $t=10$ h ($p<0.001$) after injection. Cumulative food intake was significantly reduced by 1.0 μg/kg sCT at $t=6$ h and $t=8$ h after injection ($p<0.05$) (Fig. 4B). Rats injected with 5.0 μg/kg showed a significantly reduced food intake during the entire experiment when food was present, i.e. between $t=3$ h and $t=24$ h after injection ($p<0.01$). Food intake was normalized during the wash-out period, indicating that there was no carry-over between the trials (data not shown). There was no significant effect on body temperature after

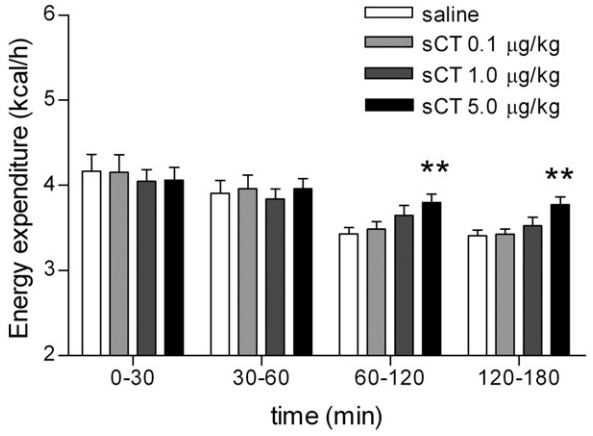

Fig. 3. Effect of sCT (0.1 μg/kg, 1.0 μg/kg or 5.0 μg/kg IP) or saline on energy expenditure. Rats ($n=10$) were injected in a randomized cross-over design in the middle of the light phase without access to food for 3 h after the injection. Data are expressed as mean±SE per time interval, normalized to kcal/h. **$p<0.01$ compared to saline.

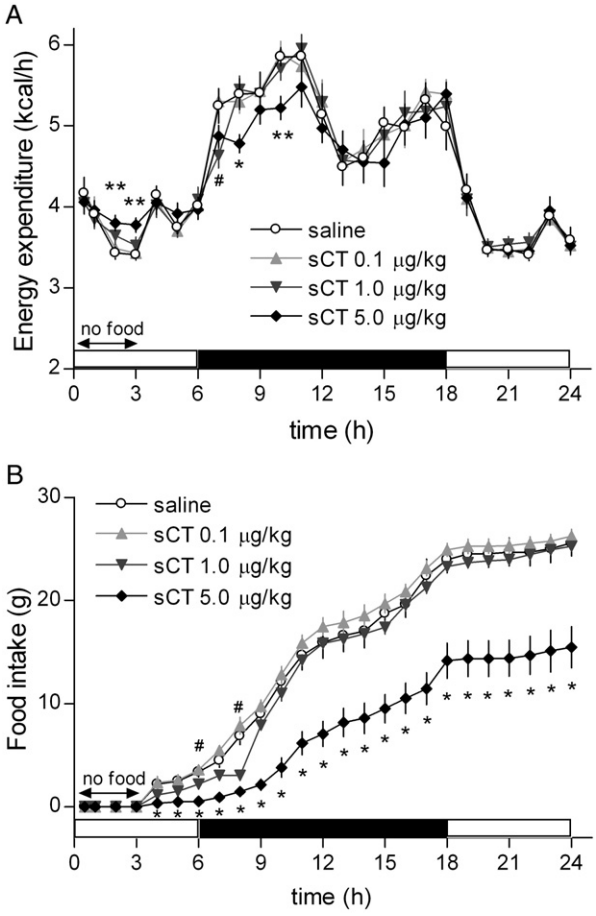

Fig. 4. A: Twenty-four hour time course of energy expenditure after injection of sCT (0.1 μg/kg, 1.0 μg/kg or 5.0 μg/kg IP) or saline. Rats ($n=10$) were injected in a randomized cross-over design in the middle of the light phase without access to food for 3 h after the injection. Data are expressed as mean±SE per hour. $^{\#}p<0.05$ sCT 1.0 μg/kg vs. saline, $^{*}p<0.05$ sCT 5.0 μg/kg vs. saline, $^{**}p<0.01$ sCT 5.0 μg/kg vs. saline. B: Twenty-four hour time course of food intake after injection of sCT (0.1 μg/kg, 1.0 μg/kg or 5.0 μg/kg IP) or saline. Rats ($n=10$) were injected in a randomized cross-over design in the middle of the light phase without access to food for 3 h after the injection. Data are expressed as mean±SE per hour. $^{\#}p<0.05$ sCT (1.0 μg/kg) vs. saline. $^{*}p<0.01$ sCT (5.0 μg/kg) vs. saline.

refeeding. Physical activity of rats treated with 5.0 μg/kg sCT was significantly lower only at $t=5$ h compared to control ($p<0.05$). At all other time points, physical activity was not significantly different (data not shown). Cumulative physical activity was not significantly different between the groups at any time point.

4. Discussion

The present study was designed to investigate the acute effect of one single bolus injection of amylin or its agonist sCT on energy expenditure in rats. Under these conditions, at a dose which significantly reduced food intake, amylin did not have a significant effect on energy expenditure when injected in rats either at dark onset with ad libitum access to food, or when injected in the middle of the light phase without access to food. On the other hand, sCT which has a longer duration of action compared to amylin [18], significantly increased energy expenditure during the period without access to food. Conversely, when food was returned, energy expenditure was decreased by sCT.

In the first experiment, amylin did not influence energy expenditure while food intake was strongly reduced during the first 2 h after administration. This reduction in food intake is in line with previous reports [6]. Reduced food intake is usually accompanied by a compensatory decrease in energy expenditure. Because there was no difference in energy expenditure in amylin-treated rats compared to control, despite the reduced food intake, this could indicate that amylin prevents the compensatory decrease in energy expenditure in animals that eat less.

To investigate the effect of amylin without the possible confounding effect of reduced food intake on energy expenditure, we repeated the experiment by injecting amylin in the middle of the light phase, without giving the rats access to food for 3 h. This time point of injection and duration of food deprivation were chosen because 3 h food deprivation in the middle of the light phase had only little influence on the energy expenditure of the control animals and because rats hardly consume any food during that period of the light/dark cycle [21]. However, as in the first experiment, amylin had no clear effect on energy expenditure or RQ. There was only a slight, non-significant increase in energy expenditure in the first 30 min after injection. It is possible that the short half-life of amylin (~13 min [22]) prevented a possible acute effect of amylin on energy expenditure after a single injection at a near-physiological dose. Further, equilibration of the atmosphere in the metabolic cage after injecting the animals might have taken too long for amylin to show a significant effect.

To circumvent this potential problem, we used sCT in the next experiment. In fact, and in contrast to amylin, one single injection of its agonist sCT (5 μg/kg) resulted in a significant increase in energy expenditure when the animals had no access to food. The anorectic effect of sCT is of much longer duration than amylin's anorectic effect [18] due to an irreversible binding of sCT to the amylin receptor [23,24]. This irreversible binding, and hence longer duration of action, may explain why sCT was able to significantly increase energy expenditure, whereas amylin had no effect. The long lasting effect of sCT was also reflected in a reduced food intake even at the time of refeeding, i.e. 3 h after injection. Most likely energy expenditure appeared to be decreased by sCT compared to controls starting 4–5 h after refeeding as a consequence of the reduced food intake. It could well be that, due to sCT's strong anorectic action, the compensatory decrease in energy expenditure in animals that eat less overruled the increase in energy expenditure by sCT.

It cannot be excluded that the dose of amylin used in these experiments was too low to have a significant effect on energy expenditure in an acute setting. The reported ED_{50} of amylin for food intake in rats is ~25 μg/kg [25], which is more than two times higher than the highest dose used in this study. Furthermore it has been shown that the doses used in this experiment have an anorectic effect mainly after 12 or 24 h fasting [4], whereas the current studies were carried out in ad

libitum fed animals. Finally, the more potent and longer lasting sCT did increase energy expenditure, which may also be an indication that at higher doses amylin may increase energy expenditure.

Chronic infusion evades the problem of short half-life of amylin and may have stronger effects on energy expenditure than one single injection. Recently, Mack et al. showed that diet induced obesity (DIO) prone rats chronically treated with amylin showed reduced food intake and body weight gain without a compensatory decrease in energy expenditure 11 weeks after the start of the infusion [26], similar to what we observed in an acute setting in experiment 1. In another study, Roth et al. found that amylin increased energy expenditure in DIO prone rats after 3 weeks of chronic treatment [19]. In the present study we used lean rats, whereas the above mentioned studies used DIO rats. Differences in body weight and adiposity certainly affect energy expenditure. Because amylin's effect on food intake is similar in lean and obese rats, it may be expected that the effect of amylin on energy expenditure is not very different between lean and obese rats. However, this needs to be tested in future experiments.

The mechanism by which amylin or sCT increases energy expenditure is unknown. One speculative possibility could be that sCT increases Na^+–K^+-ATPase activity, like amylin does [27], and thereby increases energy expenditure [28,29]. Energy expenditure is not increased due to increased physical activity, because in neither of the performed experiments an increase in physical activity was observed. Experiments 2 and 3 were performed in the middle of the light phase when spontaneous physical activity was extremely low. Furthermore, a recent publication demonstrated that amylin did not influence physical activity [30].

In the present study we did not observe any effect on body temperature, which does not support the hypothesis that hyperthermia is the underlying mechanism of stimulated energy expenditure by amylin. Two early publications reported that amylin injected centrally resulted in hyperthermia [2,17]. However, these studies both used very high doses. Recently, Roth et al. concluded that increases in energy expenditure by amylin are not likely to be the result of specific amylin induced-thermogenesis, because the increased energy expenditure was completely due to altered body composition, i.e. amylin-treated rats had more lean body mass than control rats [19]. So far, it therefore remains questionable whether amylin can increase body temperature.

In summary, a single bolus injection of a (near) physiological dose of amylin did not significantly influence energy expenditure when given at dark onset in rats that had ad libitum access to food or when given in the middle of the light phase to rats without access to food. However, the data indicate that amylin may prevent the decrease in energy expenditure that is normally seen in animals that eat less. Further, an acute injection of sCT increased energy expenditure compared to controls when the animals did not have access to food. Future studies will focus on the long-term effect on energy expenditure and body temperature by chronic infusion of amylin or sCT using osmotic minipumps.

Acknowledgement

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