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Published in:
Brain-research

ELSEVIER

Brain Research 777 (1997) 147–152

BRAIN RESEARCH

Research report

Insulin in the arcuate nucleus of the hypothalamus reduces fat consumption in rats

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Accepted 26 August 1997

Abstract

Data are accumulating that insulin acting in the central nervous system is a physiological regulator of food intake and body weight, presumably via its effect in the hypothalamus. The present study investigated whether infusion of a small dose of insulin into two major hypothalamic insulin-binding areas also has an effect on diet selection and behavior. At the beginning of the dark period, rats received local bilateral infusions of 4 μU of insulin or vehicle during 34 min into the arcuate (ARC) or paraventricular (PVN) nucleus of the hypothalamus. Consumption of carbohydrate (C)-, protein (P)-, and fat (F)-enriched food and time spent on certain behaviors (drinking, resting, grooming, rearing, exploring/sniffing) were assessed during the first nocturnal hour. In addition, 21-h diet selection was assessed. The percentage contribution of macronutrients (C/P/F) to total energy content of the C-, P-, and F-enriched diets was 71.9/17.2/10.9, 45.8/43.4/10.8, and 47.1/17.5/35.4, respectively. During the first hour, infusion of insulin into the PVN increased grooming behavior compared to infusion of the vehicle. Although infusion of insulin had no effect on diet selection during the first hour, insulin infused in the ARC caused a reduction in F-enriched food consumption and total intake of F (as a macronutrient) over the 21-h period without altering total food intake. Infusion of a higher dose of insulin (10 μU) into the third ventricle had no effect on any of the assessed parameters. The data are explained to indicate that insulin (being an indicator of a positive energy balance) adjusts body weight homeostasis by modulating the preference for fat, at least at the level of the ARC, but not at the PVN. © 1997 Elsevier Science B.V.

Keywords: Hypothalamus; Infusion; Diet selection; Fat; Carbohydrate; Protein; Enriched; Macronutrient

1. Introduction

Insulin secretion is elevated during and after food intake, and it has a major function in eliminating absorbed fuels from the blood by stimulating the storage of these fuels in tissue depots [38,42]. Insulin is also believed to play a role in the regulation of food intake and body weight homeostasis by acting in the central nervous system [43,44]. Insulin binding-sites have previously been identified in a number of brain areas [6,13,18], including the paraventricular (PVN) and arcuate (ARC) hypothalamic nuclei, areas involved in the control of food intake and body weight homeostasis [5]. Supportive of the premise that insulin serves as a feedback regulator of food intake to control body weight are numerous reports that administration of insulin into hypothalamic areas or into cerebral ventricles causes reliable and predictable decreases in food intake and body weight [4,9,10,19,24,25,27,29,44] without causing discomfort or nausea [11]. Furthermore, infusion of insulin antibodies into the ventromedial hypothalamic area results in increased food intake [36].

Recently, we have observed that chronic administration of insulin (6 mU/day) into the third ventricle (3V) of rats that had continuous access to pure macronutrients selectively inhibited fat (F) consumption without altering carbohydrate (C) or protein (P) consumption [12]. To investigate further the effects of central insulin on food selection and behavior, a low dose of insulin (4 μU over 34 min) was bilaterally infused at the beginning of the dark period into two major hypothalamic insulin-binding sites (the PVN and ARC) of rats that were freely choosing from three diets that were enriched in their C, P, or F content relative to the other macronutrients. Diet selection over the first hour in the dark period as well as over a 21-h period was assessed. In addition to the effects of insulin on diet

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preference during the first hour, the effects of insulin infusion into the PVN or ARC on other behaviors (grooming, rearing, exploring, resting, and drinking) was investigated. To investigate whether leakage of insulin into the ventricular system could attribute to the results obtained by infusion of insulin into hypothalamic sites, the effect of infusion of a higher dose of insulin (10 μU) into the 3V on 1-h and 21-h diet selection and behavior (only assessed during the first hour of the dark phase) was investigated as well.

2. Materials and methods

2.1. Animal preparation

Male Wistar rats weighing 360–390 g at the beginning of the experiments were used. The animals were individually housed in Plexiglas cages (25 × 25 × 30 cm) at normal ambient temperature (20 ± 2°C), and had continuous access to lab chow (Hope Farms) and water unless otherwise stated. The rats were maintained on a 12:12 h light/dark regime (00.30–12.30 h: lights on), and they were handled and weighed every day at 09.00 h.

Brain guide cannulas were made of 27-gauge (Braun Melsungen) disposable injection needles (length 19.2 mm). The blunt ends were positioned (for 5 mm) and soldered inside 22.25-gauge stainless-steel 1-cm sleeves. Obturators were placed inside the guide cannulas and were cut to terminate at the same point as the guide cannulas. During halothane/O_2/N_2 anesthesia, the skin covering the skull was incised in the midline and a 2 × 2-mm craniotomy was performed using a high-speed drill. With the aid of a stereotaxic apparatus (Narashige, SR-6, SM-11 micromanipulator), the sterile pre-constructed cannulas with obturators were bilaterally positioned with their tips 1 mm above the arcuate (ARC, $n = 8$) or the paraventricular nucleus (PVN, $n = 8$). The beveled ends of the guide cannulas were medially oriented. In a third group of rats, a single cannula was positioned so its tip was located 1 mm above the center of the third ventricle (3V, $n = 6$). The sagittal sinus was shortly displaced with a metal probe while the cannula was lowered towards the 3V. The coordinates for PVN-, ARC- and 3V-cannula placements were found using the atlas of Paxinos and Watson [26]. The lateral coordinates (PVN: 0.5 mm; ARC: 0.4 mm; 3V: 0 mm) were found by assigning the sagittal sinus as zero. The anteroposterior coordinates were found by using bregma and the inter-aural line as points of reference (caudal to bregma, PVN: 2.0 mm; ARC: 3.6 mm; 3V: 2.0 mm). The dorsoventral coordinates were found by using the dura as zero (PVN: 7.4 mm; ARC: 9.0 mm; 3V: 8.2 mm). Whenever the weights of the animals differed from those used in the atlas, or the dorsoventral and anteroposterior coordinates of bregma and interaural line differed from distances in the atlas, the coordinates were adjusted according to the method of Paxinos and Watson [26]. Brain cannulas were fixed to the skull with sterile anchor screws and dental acrylic. The exteriorized cannula ends and obturators were protected by polyethylene caps. An aluminum screwable cap covered cannulas, caps, and obturators. The skin incision was closed with surgical sutures so that only the aluminum cap protruded outside the skin.

2.2. Experimental procedures

After surgery, the animals were put on a diet from which they could freely select carbohydrate (C)-enriched, protein (P)-enriched, and fat (F)-enriched food in separate containers (diameter: 3 cm; height: 1.5 cm). The percent contributions of macronutrients (C/P/F) to total energy content of the C-, P-, and F-enriched diets were 71.9/17.2/10.9, 45.8/43.4/10.8, and 47.1/17.5/35.4, respectively. The constituents of the diets are presented in Table 1. The caloric densities of the diets were chosen so that rats on average selected roughly the same amount of each diet on a daily basis. The enriched foods were prepared daily from a frozen stock and were presented to the animals 3 h before lights off. The containers were randomly placed onto a wire mesh which was put over the bottom of the cages to allow correction for spillage. When the rats had reacquired and surpassed their preoperative weights (at least 1 week following surgery), daily intake of the enriched diets was assessed for each rat. The experiments started when the rats displayed a constant intake of the diets for 1 week. To avoid stress of novelty, including being connected to brain infusion tubing and assessment of behavior, all rats were habituated to the experimental procedures three times on separate days.

2.3. Infusion procedures

Insulin (Actrapid NOVO) was dissolved in sterile synthetic cerebrospinal fluid (sCSF) containing 127.64 mM NaCl, 2.55 mM KCl, 1.26 mM $CaCl_2$, 0.93 mM $MgCl_2 \cdot H_2O$ and 0.05% bovine serum albumin (to prevent adhesion of insulin to vials and tubing) to concentrations of 0, 1 and 5 μU of insulin per μl of sCSF. The pH of the sCSF was 7.4. After preparation, the solutions were divided into

Table 1
Composition of carbohydrate (C)-, protein (P)-, and fat (F)-enriched diets

Diets	C-enriched	P-enriched	F-enriched
Lab chow powder (Hope Farms)	750	750	750
Glycerol (85PC Ph. Eur.)	100	100	100
D(+)-Glucose (Merck)	250	–	–
Caseine (Merck)	–	250	–
Vegetable fat (AH, Netherlands)	–	–	100
Cellulose (PH101 Ph. Eur.)	–	–	250
Water	300	900	800
Caloric density (kcal/g)	2.745	2.148	1.903

Constituents of diets are expressed in grams.

Table 2
Effect of administration of insulin or synthetic cerebrospinal fluid (sCSF) into the paraventricular hypothalamic nucleus (PVN), the arcuate nucleus (ARC), or the third ventricle (3V) on duration (in s) of different behaviors during the first hour of the dark period

	PVN ($n = 6$)		ARC ($n = 7$)		3V ($n = 5$)	
	Insulin	sCSF	Insulin	sCSF	Insulin	sCSF
Drinking	88 ± 46	116 ± 45	36 ± 16	24 ± 14	44 ± 25	46 ± 20
Resting	755 ± 273	1056 ± 216	1723 ± 263	1409 ± 334	1640 ± 327	1714 ± 225
Grooming	1436 ± 161*	1035 ± 119	725 ± 32	742 ± 122	807 ± 158	793 ± 112
Rearing	414 ± 108	383 ± 77	240 ± 49	82 ± 31	184 ± 57	180 ± 37
Sniffing/exploring	617 ± 136	638 ± 87	271 ± 100	148 ± 62	237 ± 57	303 ± 61

Insulin (2 μU into each PVN or ARC, or 10 μU into the 3V) or sCSF was infused (2 μl) over a 34-min period which started immediately following lights off.
* $P < 0.05$.

several portions and stored at −80°C until 2 h before infusion. One hour before the dark period on experimental days, the obturators were replaced by injection cannulas that were connected to infusion tubing (length: 0.4 m; o.d.: 0.6 mm; i.d.: 0.2 mm). Infusion tubing and injection cannulas were completely filled with the infusate before insertion. An injection cannula consisted of fused silicate (LC Service, the Netherlands; o.d.: 0.150 mm; i.d.: 0.075 mm) which was, at one end, glued (Cyanolyt, 3M) inside the metal sleeve of a 27-gauge needle (length: 1 cm). The metal sleeve tightly fitted inside the polyethylene infusion tubing. After insertion, the injection cannulas extended 1 mm beyond the guide cannulas so that they reached the dorsal aspects of the ARC, the PVN, or the center of the 3V. The injection cannulas were held in place by an aluminum screwable cap with a small hole to pass the infusion tubing. At the other end, each tube was connected to a 10-μl Hamilton syringe that was mounted on an infusion pump which provided a constant flow of 0.058 μl/min.

2.4. Experiments

Food containers were filled with fresh food 3 h before lights off and presented to the rats (the same as all other days) and were reweighed just before lights off (a red light was left on for practical reasons). At lights off, 2 μl of sCSF or insulin was administered through each cannula into each PVN, ARC or 3V over a 34-min period. The animals received insulin (PVN and ARC: 1 μU/μl; 3V: 5 μU/μl) or sCSF in a counterbalanced order, with 1 week between successive experiments. Infusion of a higher concentration of insulin into the 3V was given to investigate whether leakage from neuropile into the 3V could influence diet selection and/or behavior. During the first nocturnal hour, time (in s) spent on different behaviors (drinking, grooming, resting, rearing, and exploring/sniffing) of the animals was assessed continuously by an investigator who was blind to the experimental condition (infusion of insulin or sCSF). The period of 1 h was chosen because the largest effects of putative anorectic compounds to alter food intake behavior are usually observed within 1 h, even when delivered into the cerebroventricular system [34]. After that hour, injection cannulas were replaced by obturators and food intake of the three enriched diets was assessed. The next day (9 h in the following light phase and 21 h after infusion), food intake was assessed again. Intake from containers was corrected for evaporation.

2.5. Histology

A few days after the last experiments, rats were anesthetized with pentobarbital (0.1 ml/kg, i.p.) and obturators were replaced by injection cannulas filled with a dye (Chicago Sky Blue). A volume of 0.2 μl was infused to mark infusion spots. Then brains were taken from crania after the rats had been perfused with heparinized saline and 4% formaldehyde. Brains were stored for 1 week in

Table 3
Effect of administration of insulin or synthetic cerebrospinal fluid (sCSF) into the paraventricular hypothalamic nucleus (PVN), the arcuate nucleus (ARC), or the third ventricle (3V) on consumption (in kcal) of carbohydrate (C)-, protein (P)-, and fat (F)-enriched diets during the first hour of the dark period

	PVN ($n = 6$)		ARC ($n = 7$)		3V ($n = 5$)	
	Insulin	sCSF	Insulin	sCSF	Insulin	sCSF
CH-rich	5.35 ± 1.02	5.76 ± 1.92	2.78 ± 1.13	2.78 ± 0.98	3.54 ± 1.29	3.38 ± 1.39
P-rich	2.00 ± 0.83	1.58 ± 0.79	1.41 ± 0.72	2.06 ± 1.71	1.12 ± 0.62	0.82 ± 0.73
F-rich	0.22 ± 0.20	0	1.69 ± 0.61	1.55 ± 0.50	1.22 ± 1.09	1.56 ± 1.27
Total	7.60 ± 1.08	7.34 ± 1.62	5.88 ± 1.36	6.39 ± 1.63	5.88 ± 1.14	5.57 ± 2.01

Insulin (2 μU into each PVN or ARC, or 10 μU into the 3V) or sCSF was infused (2 μl) over a 34-min period which started immediately after lights off.

Table 4
Effect of administration of insulin or synthetic cerebrospinal fluid (sCSF) into the paraventricular hypothalamic nucleus (PVN), the arcuate nucleus (ARC), or the third ventricle (3V) on consumption (in kcal) of carbohydrate (C)-, protein (P)-, and fat (F)-enriched diets during a 21-h period

	PVN ($n = 6$)		ARC ($n = 7$)		3V ($n = 5$)	
	Insulin	sCSF	Insulin	sCSF	Insulin	sCSF
CH-rich	35.2 ± 5.4	30.9 ± 6.6	38.5 ± 7.5	30.1 ± 3.1	42.4 ± 13.6	39.9 ± 5.1
P-rich	21.2 ± 5.3	25.8 ± 8.3	33.3 ± 7.7	33.2 ± 9.0	14.1 ± 5.9	20.0 ± 5.2
F-rich	15.2 ± 3.1	17.1 ± 5.4	13.2 ± 4.0 *	23.7 ± 5.7	19.3 ± 7.2	24.2 ± 5.8
Total	71.5 ± 2.3	73.7 ± 2.1	85.0 ± 6.0	86.9 ± 5.5	75.8 ± 9.1	84.2 ± 4.9

Insulin (2 μU into each PVN or ARC, or 10 μU into the 3V) or sCSF was infused (2 μl) over a 34-min period which started immediately after lights off.
* $P < 0.01$.

4% formaldehyde and 1 day in 30% sucrose. Brains were quickly frozen and cut on a cryostat microtome. The slices were stained with a 1% cresyl fast violet dye and examined under a light microscope. Only rats with the tip of the cannula in the PVN ($n = 6$), ARC ($n = 7$) or 3V ($n = 5$) were included in the analyses.

2.6. Data analyses

A two-way analysis of variance (ANOVA) with drug (within) and order of application (between) as factors was applied to determine significant differences between the effects of insulin and sCSF on total food intake, diet selection and different behaviors during the first hour of the dark period and food intake and diet selection and total food intake over a 21-h period. A probability level of $P < 0.05$ was taken for statistical significance. Data are expressed as means ± standard error of the mean.

3. Results

3.1. Behavior during the first nocturnal hour

The effects of infusion of insulin and sCSF into the PVN, ARC, or 3V on time spent on different behaviors are presented in Table 2. ANOVA revealed a significant effect of insulin in the PVN on time spent on grooming during the first nocturnal hour ($F_{1,4} = 18.94$, $P = 0.012$). There was no effect of the order of drug administration. None of the other behaviors were significantly affected by insulin in the PVN. Insulin infused into the ARC or 3V did not affect any of the assessed behaviors during the first hour of the dark phase.

3.2. Diet selection

Infusion of insulin into the PVN, ARC, or 3V of rats did not significantly alter 1-h consumption of the enriched diets, absolute intake of separate macronutrients from the three enriched diets, or total consumption (see Table 3). Over the 21-h period (which included the whole dark phase and 9 h of the following light phase), infusion of insulin into the PVN, ARC, 3V did not affect total caloric consumption (Table 4). However, infusion of insulin into the ARC significantly affected consumption of the F-enriched diet over the 21-h period ($F_{1,5} = 18.55$, $P = 0.008$). In addition, insulin infused in the ARC had an effect on the total consumption of F (as a macronutrient) taken from all three diets (12.5 ± 1.1 kcal vs. 15.2 ± 1.6 kcal as fat, $F_{1,5} = 17.92$, $P = 0.008$). Finally, insulin in the ARC affected the percentage of calories consumed as F from total (C, P, and F) during the 21-h period (from $17.71 \pm 1.57\%$ to $14.96 \pm 1.27\%$, $F_{1,5} = 14.99$, $P = 0.012$). Again, there was no effect of the order of drug administration.

4. Discussion

The present experiments were designed to investigate the effects of administration of insulin at the beginning of the dark phase into selected hypothalamic areas on short- (1 h) and long-term (21 h) behavior and diet selection. The most important result of the present study is the finding that insulin infused into the arcuate nucleus (ARC), but not into the paraventricular nucleus of the hypothalamus (PVN) reduced fat (F) consumption over a 21-h period without having an effect on total food intake. The reduction in F consumption was partly compensated by an increased (but non-significant) intake of the carbohydrate (C)-enriched diet. The effects of insulin on diet selection could not be attributed to leakage of insulin from hypothalamic neuropile into the 3V and subsequent acting on more peripheral tissue, because infusion of a higher dose of insulin (10 μU) directly into the 3V did not have an effect on any of the assessed variables. Thus, the insulin effects in the present experiments are the result of local action on neural structures.

During the first hour of the dark phase, only insulin infused into the PVN had an effect on behavior; i.e. it caused an increase in time spent on grooming. Although we do not have an explanation for this effect, it is important to note that the PVN (relative to other regions in the hypothalamus) is a very sensitive site for stimulation of

grooming behavior [16,40]. The data of the present study are consistent with that since infusion of the vehicle per se (which consisted of isotonic synthetic cerebrospinal fluid with a pH of 7.4) into the PVN caused dramatically more grooming than infusion of the vehicle in the ARC or 3V, an effect that was even further augmented by insulin.

The results of the present study are consistent with our previous observation that chronic infusion of insulin over several days into the 3V reduced F ingestion [12]. While caution must be exercised when using diet selection paradigms [28,41], it may be suggested that the reduction in F consumption was caused by a modulatory effect of insulin on the synthesis and/or secretion of hypothalamic transmitters that are involved in diet selection (for review see [8,22]). It has been reported that the level of galanin in the PVN is well correlated to F consumption and inversely correlated to plasma insulin levels [1]. Thus, it may be possible that insulin, at the level of the ARC, reduces galanin synthesis in neurons that project to the PVN, and this could have caused a reduction in F consumption. This presumed relationship between insulin and hypothalamic galanin would only have relevance at higher levels of insulin, such as during prandial and post-prandial periods, since food deprivation (which results in a reduction in the level of plasma insulin) has no effect on galanin levels and/or synthesis [7,33]. There is a well-known inhibitory effect of insulin on synthesis of neuropeptide Y (NPY) in the ARC [31]. However, hypothalamic transmission of NPY mainly seems to regulate total food intake with emphasis on consumption of C instead of F [2,20,23,35].

A different explanation for insulin's effect on diet consumption is based on the observation that insulin is not effective at all times during the day to reduce food intake [27]. It may be that there is a temporal window during the dark phase when it can affect food intake. Rats consume relatively large amounts of C at dusk, whereas F is mainly consumed at a later period in the dark phase [37,39]. Consistent with those observations is the finding in the present study that rats favored the C-enriched diet above the other diets during the first nocturnal hour in the present study. Thus, assuming that the rats in the present study were nocturnally selecting the diets in the reported pattern, it is possible that insulin in the ARC was most efficacious in causing a reduction in food intake at the time when the rats would be predominantly selecting F. A factor previously described to attenuate insulin's efficacy to reduce food intake is corticosterone (for review see [14]). Since the level of plasma corticosterone peaks at the beginning of the dark phase [3,15], it is possible that the sensitivity for central insulin is at its nadir at that time, explaining the relative insensitivity for centrally infused insulin.

In summary, infusion of insulin into the ARC, but not into the PVN, reduces 21-h F consumption without affecting total food intake in rats. This effect may be based on insulin's ability to affect the synthesis and/or transmission of hypothalamic neurotransmitters, such as galanin. Alternatively or additionally, insulin's effect on food intake may be limited to the period when F is predominantly preferred, i.e. during the late dark period. Since dietary F produces less satiety relative to other macronutrients, it increases total intake and induces more F deposition and a positive energy balance [17,30]. In healthy nutritionally stable subjects, however, body adiposity remains relatively constant over longer periods of time [21]. Thus, the data in the present study intuitively fit the hypothesis that insulin, being an indicator of body adiposity, in addition to the newly discovered Ob-protein [45], serves as a feedback signal to the CNS [32,34,43] to regulate the consumption of F.

Acknowledgements

This study was supported by grants from the Dutch Scientific Organization, The Dutch Diabetes Foundation, and the N.I.H. (DK-17844). S.C. Woods and T.E. Thiele are thanked for discussing the manuscript.

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