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Immediate but not long-term intranasal administration of insulin raises blood pressure in human beings

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Abstract

Intranasal administration of insulin has been shown to influence neuroendocrine functions via an effect on central nervous mechanisms. Because insulin, in particular when infused into cerebral arteries, induces blood pressure (BP) elevation by an unknown mechanism, we investigated whether insulin exerts similar effects on BP after intranasal administration. To evaluate the immediate effects of insulin on BP, 20 IU of human insulin was intranasally administered every 10 minutes over a 2-hour period. Blood pressure, heart rate, and muscular sympathetic nervous activity (MSNA) were continuously monitored. For evaluating the effects of subchronic administration of insulin, changes during and after 8 weeks of treatment with 160 IU insulin/d on BP were monitored. Compared with placebo, the immediate nasal administration of insulin raised diastolic BP (12.21% ± 5.10%; $P < .05$), mean arterial BP (10.81% ± 4.32%; $P < .04$), and systolic BP (9.53% ± 4.66%; $P < .08$), whereas MSNA and heart rate were unaffected. In contrast, prolonged intranasal insulin administration did not affect BP ($P > .62$ for all comparisons). The immediate increase in BP in the face of an unsuppressed MSNA after insulin suggests that intranasal insulin transiently changes the baroreflex set point. Thus, data suggest that intranasal insulin administration affects BP regulatory centers in the brain. However, the effect is not observed with prolonged administration of the hormone, suggesting the emergence of counterregulatory processes.

1. Introduction

Insulin is an important neuromodulator in the central nervous system [1-6]. Systemic insulin enters the brain primarily via a receptor-mediated transport system located in endothelial cells of brain microvessels [7-9]. Highest densities of brain insulin receptors are found in the hippocampus, the hypothalamus, and throughout the limbic system [10,11]. Elevation of central nervous insulin levels affects hypothalamic structures involved in body weight regulation [12-14]. In animals, intracerebroventricular administration of insulin reduces food intake and body weight, and a neuron-specific disruption of the insulin receptor gene (NIRKO mice) has reversed effects [8,11,15,16]. Furthermore, the enhancing effects of insulin on central nervous memory processing are well established [17,18]. Intracerebroventricular infusion of insulin improves hippocampus-dependent memory formation in a passive avoidance task [17] whereas intracerebroventricular administration of streptozotocin, a substance that is known to induce diabetes, impairs learning and memory functions in adult rats [19]. In human beings, intravenous infusion of insulin substantially improved hippocampus-dependent (declarative) memory performance [20]. This effect might be of clinical relevance because the severity of symptoms has been found to be correlated with a distinct decrease in brain insulin concentration in Alzheimer disease [21].

In human beings, long-term intravenous infusion of insulin to improve memory function or to induce catabolic effects is not feasible because of its hypoglycemic action. To prevent such systemic side effects of bioactive compounds, the transnasal route as a noninvasive and efficacious method of drug delivery enables a direct access of peptides to the brain without substantial absorption into the blood stream

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[22-29]. After intranasal administration, substances such as insulin enter the cerebrospinal fluid from the nasal mucosa via intercellular clefts along the bulbus olfactorius and nervus olfactorius [29-31]. Because insulin after intranasal administration does not enter the circulation in substantial amounts, this route of administration may be useful in the long-term therapeutic manipulation of neuroendocrine functions. Previous studies have provided evidence that long-term intranasal administration of insulin enhances hippocampus-dependent memory performance and can also reduce body fat stores [32,33].

Insulin has substantial effects on blood pressure (BP) regulation, which consists mainly in vasodilatation and sympathoexcitation. The sympathoexcitation is possibly in part the result of a baroreflexive response to the vasodilatory effect of insulin on peripheral blood vessels [34]. However, animal experiments have indicated that central nervous insulin can induce sympathoexcitation via central nervous autonomous centers as well [35-38]. Thus, hypertension after insulin administration can be a consequence of a sympathoexcitatory effect in the brain that causes vasoconstriction and remains unopposed by peripheral vasodilatation in insulin-resistant subjects [39-42]. On this background, the question arises whether the intranasal administration of insulin would likewise affect brain sympathetic nervous centers and consequently increase BP. To answer this question, we examined immediate and long-term changes in muscular sympathetic nervous activity (MSNA) and BP after intranasal administration of insulin.

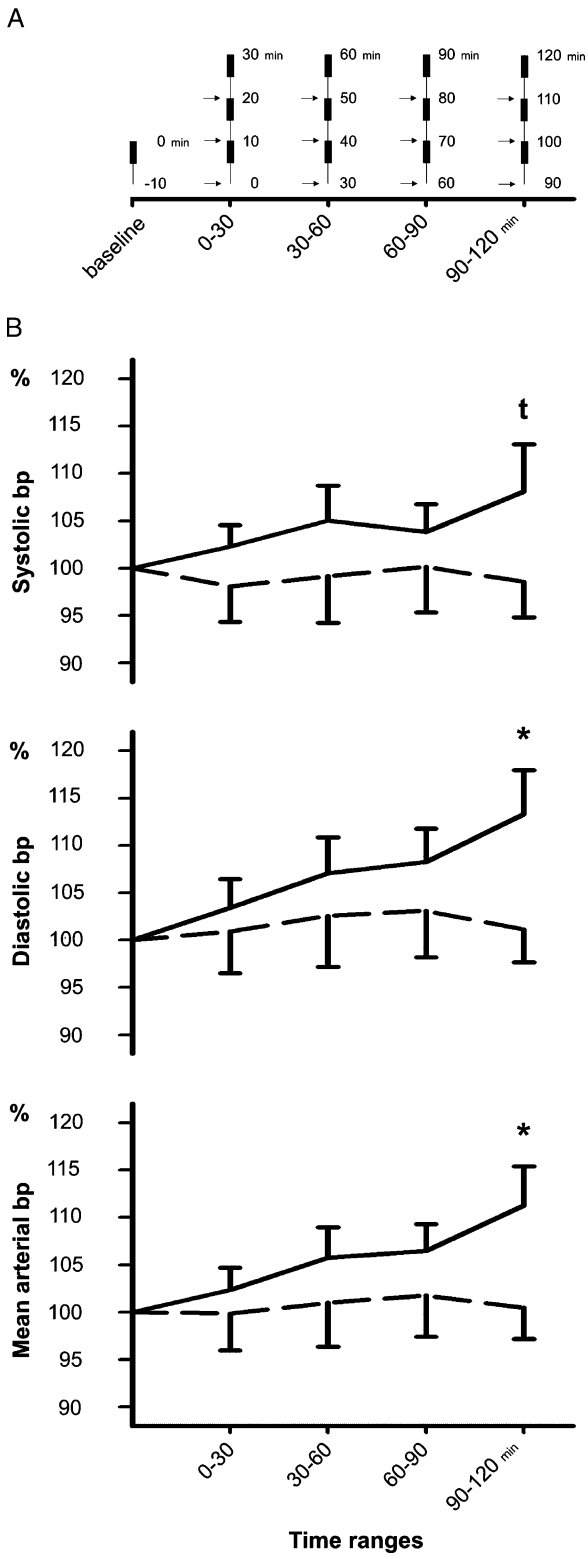

Fig. 1. Immediate insulin experiment. A, Procedure. After a 10-minute baseline period, 8 subjects were intranasally administered either 0.2 mL (20 IU) of insulin or vehicle every 10 minutes over a 110-minute period, amounting to a total dose of 240 IU of human insulin (arrows indicate time of substance administration). Blood pressure, heart rate, and MSNA were evaluated during the last 5 minutes of the baseline period and during the second 5-minute interval after each intranasal administration (vertical thick lines, time interval of measurement). For analysis, average values of 3 consecutive intervals were compared. B, Mean (±SEM) systolic BP, diastolic BP, and mean arterial BP during the insulin (solid line) and placebo (dashed line) conditions. Values are presented in % as derived from the Finapres device with the baseline set to 100%. Significant differences between the effects of placebo and those of insulin are indicated (*$P < .05$; $^{t}P < .1$).

2. Subjects and methods

The double-blind placebo-controlled experiments were performed in healthy, normal-weight (body mass index [BMI], <25 kg/m^2), and nonsmoking subjects. Subjects were instructed not to eat and drink during the 8 hours preceding experimental sessions. Throughout the sessions, subjects remained in a supine position. For repeated blood sampling, a polyvinyl catheter was inserted into a forearm vein in the beginning of each session. The study was approved by the local ethics committee and the volunteers gave written informed consent before participating.

2.1. Immediate effects of insulin

The experiment was designed according to a within-subject crossover comparison. Eight men ([mean ± SEM] age, 24.8 ± 0.5 years; BMI, 22.6 ± 1.7 kg/m^2) participated in

two sessions (insulin and placebo) that were conducted at least 8 days apart, with the order of conditions balanced across subjects. In one condition, subjects received insulin (Insulin Actrapid HM, Novo Nordisk, Mainz, Germany). In the other condition, they received the vehicle (HOE 31 dilution buffer for H-Insulin, Aventis Pharma, Bad Soden, Germany). After a baseline period of 10 minutes, 0.2 mL of insulin (containing 20 IU) or vehicle was intranasally administered every 10 minutes over a 110-minute period (Fig. 1A), amounting to 2.4 mL (240 IU) of insulin. Blood pressure was recorded via photoplethysmography (Finapres, Ohmeda Monitoring System, Englewood, Colo) throughout the session, starting 10 minutes before the first nasal puff (baseline) and ending 2 hours later. The Finapres device enables continuous and noninvasive measurement of relative changes in arterial BP. In addition, heart rate (via electrocardiogram) and MSNA were registered. The MSNA was continuously measured by microneurography of the peroneal nerve as described elsewhere [43]. This method allows direct recordings of summed potentials of sympathetic neurons to the vascular bed of the skeletal muscle [44,45] and correlates with plasma norepinephrine concentrations and norepinephrine spillover [46]. Sympathetic bursts were identified by inspection of the mean voltage neurogram and quantified as bursts per minute. To monitor the secretory activity of the hypothalamus-pituitary-adrenocorticoid (HPA) axis in the immediate insulin study, blood samples for the determination of corticotropin (ACTH) plasma concentrations were taken at the beginning of the baseline phase (−10 minutes; Fig. 1A) and at the end of the treatment period (120 minutes; Fig. 1A). Samples were centrifuged immediately, and the plasma was stored at −20 °C until assay (Pharmacia Insulin RIA100, Pharmacia & Upjohn Inc, Uppsala, Sweden).

2.2. Subchronic effects of insulin

Subjects were randomly assigned to 2 experimental groups (each including 8 men and 8 women), an insulin group and a placebo group. Groups were comparable regarding mean age and BMI in the pretesting examination ([mean ± SEM] insulin, 24.6 ± 1.3 years and 22.6 ± 0.3 kg/m^2; placebo, 25.8 ± 1.2 years and 22.7 ± 0.4 kg/m^2). During a 2-week baseline phase, all subjects received placebo. During the following 8 weeks of treatment, subjects were intranasally administered either insulin or placebo 4 times a day: in the morning, around noon, in the evening, and before going to bed. Each dose consisted of 0.4 mL (40 IU) of insulin or vehicle (for specifications, see immediate insulin study) administered within 4 puffs of 0.1 mL (2 per nostril), amounting to 1.6 mL (160 IU) of insulin or vehicle per day. Sprays were stored at 2°C to 7°C and were replaced by new substance every 7 days. To ensure compliance, subjects kept a protocol on their intake routine. Blood pressure and heart rate were measured at the beginning of the baseline and at the end of the 8-week treatment period. Blood pressure and heart rate were recorded oscillometrically with a BP monitor (Boso Prestige, Bosch & Sohn, Jungingen, Germany) 1 hour after the administration of 0.4 mL of placebo. Muscular sympathetic nervous activity and ACTH were not measured in the subchronic insulin experiments.

2.3. Glucose levels

To control for stable plasma glucose levels after intranasal insulin administration, blood was drawn at the beginning of the baseline and every 30 minutes in the immediate insulin experiments (starting from the onset of the treatment period; 0 minute) and once a week (in the morning after an overnight fast of 8 hours) in the subchronic insulin experiments. Plasma glucose levels were determined by the hexokinase method (Aeroset, Abbott, Wiesbaden, Germany).

2.4. Statistical analyses

Data are shown as mean ± SEM. For the immediate insulin experiments, time windows selected for analysis covered the baseline and the last 5 minutes of each 10-minute period after each intranasal administration. Values of BP, heart rate, and MSNA were averaged over 3 consecutive 5-minute postadministration periods (Fig. 1A). Values were baseline adjusted by subtracting the mean value during the 10-minute baseline from the posttreatment values. Baseline-adjusted values were subjected to 2-way repeated-measures analyses of variance (ANOVA; within-subject factors: substance, time). In case ANOVA yielded a significant main effect, differences at single time intervals were specified by pairwise *t* tests. For the subchronic insulin study, BP and heart rate in the 2 conditions were compared using analyses of covariance (factor: treatment), with the values of the baseline sessions serving as covariates. Generally, a *P* value less than .05 was considered significant.

3. Results

3.1. Immediate effects of insulin

In the immediate experiments, intranasal insulin slightly increased BP compared with the effects of placebo. However, this effect did not reach significance until 95 to 120 minutes after the start of the intranasal administrations (Fig. 1B). During this interval, the relative increase in diastolic BP and mean arterial BP compared with the respective baseline levels ([insulin vs placebo] systolic BP, 152.00 ± 6.25 vs 160.65 ± 8.81 mmHg; diastolic BP, 91.70 ± 7.00 vs 99.90 ± 6.72. mmHg; mean arterial BP, 110.00 ± 6.20 vs 117.40 ± 6.90 mmHg; $P > .51$ for all comparisons) averaged 14.31% ± 4.5% and 12.29% ± 3.80% on the insulin condition and 0.16% ± 2.99% and 0.46% ± 3.14% on the placebo condition, resulting in a significant difference in diastolic BP and mean arterial BP of 12.21% ± 5.10% ($P < .05$) and 10.81% ± 4.32% ($P < .04$), respectively (Fig. 1B). The changes in systolic BP observed during the 95-minute to 120-minute period after insulin administration only approached statistical significance (difference between insulin and placebo,

9.53% ± 4.66%; $P < .08$; Fig. 1B). Heart rate, MSNA bursts frequency, and plasma concentrations of ACTH were not changed by immediate intranasal insulin administration as compared with the placebo condition (Table 1).

3.2. Effects of subchronic insulin treatment

The 8-week intranasal administration of insulin did not elevate BP in comparison with the placebo condition (systolic BP, 115.29 ± 4.54 vs 115.67 ± 3.81 mmHg; diastolic BP, 68.64 ± 1.91 vs 65.67 ± 1.82 mmHg; mean arterial BP, 88.72 ± 2.46 vs 87.07 ± 2.42 mmHg; $P > .62$ for all comparisons) (Table 2). Also, no differences in heart rate were observed after subchronic insulin administration when compared with placebo (70.83 ± 2.75 vs 66.71 ± 2.57 bpm; $P > .61$).

3.3. Plasma glucose

Consistent with previous studies [3,22], ANOVA did not reveal any changes in plasma glucose concentrations after immediate ($P > .44$; see also Table 1) or subchronic ($P > .55$) intranasal administration of insulin.

Table 1
Mean (±SEM) MSNA, heart rate, plasma ACTH, and plasma glucose levels during immediate intranasal insulin administration

	Insulin	Placebo	P
MSNA (bursts/min)			
Baseline	15.33 ± 2.45	13.13 ± 1.96	
05-30 min	15.75 ± 1.48	14.97 ± 1.50	NS
35-60 min	17.36 ± 1.52	18.39 ± 1.88	NS
65-90 min	19.32 ± 1.78	18.55 ± 2.03	NS
95-120 min	20.29 ± 1.76	19.23 ± 2.20	NS
Heart rate (bpm)			
Baseline	59.91 ± 2.10	63.11 ± 2.72	
05-30 min	60.47 ± 1.06	63.77 ± 1.64	NS
35-60 min	61.39 ± 1.13	64.12 ± 1.58	NS
65-90 min	61.67 ± 0.98	63.54 ± 1.73	NS
95-120 min	62.01 ± 0.99	63.67 ± 1.51	NS
ACTH (pg/mL)			
−10 min	15.41 ± 0.53	16.60 ± 1.65	
120 min	14.76 ± 2.13	14.84 ± 1.61	NS
Plasma glucose (mg/dL)			
−10 min	87.29 ± 2.29	86.46 ± 3.65	
0 min	87.25 ± 1.74	84.75 ± 4.69	NS
30 min	86.50 ± 4.26	85.00 ± 4.50	NS
60 min	88.13 ± 2.09	89.63 ± 2.21	NS
90 min	84.38 ± 3.70	88.50 ± 2.07	NS
120 min	83.25 ± 2.40	84.25 ± 5.55	NS

After a 10-minute baseline phase, 8 subjects were intranasally administered 0.2 mL of insulin (20 I.U) or placebo every 10 minutes over a 110-minute treatment period. To assess the effects of intranasal insulin treatment on plasma ACTH levels, blood was drawn at the beginning of the baseline phase and at the end of the treatment period. Also, plasma glucose levels were measured at the beginning of the baseline (−10 minutes) and every 30 minutes starting from the onset of the treatment period (0 minute). Data on MSNA and heart rate were recorded during the 5- to 10-minute interval after the onset of the baseline phase and during the 5- to 10-minute interval after an intranasal insulin administration. For statistical analysis, values were baseline adjusted by subtracting the mean value during the 10-minute baseline from the posttreatment values. Data are expressed as mean ± SEM. Right column indicates significant differences ($P < .05$) between the effects of treatments. NS indicates nonsignificant ($P > .1$).

Table 2
Effects of 8 weeks of intranasal insulin administration on BP (mmHg) in human beings

	Insulin	Placebo	P
Baseline period			
Systolic BP	119.94 ± 3.70	120.33 ± 3.23	NS
Diastolic BP	70.31 ± 1.76	70.13 ± 1.44	NS
Mean arterial BP	91.68 ± 2.21	91.69 ± 1.55	NS
Treatment period			
Systolic BP	115.29 ± 4.54	115.67 ± 3.81	NS
Diastolic BP	68.64 ± 1.91	65.67 ± 1.82	NS
Mean arterial BP	88.72 ± 2.46	87.07 ± 2.42	NS

Baseline period–Both groups (insulin and placebo) were treated over 2 weeks with placebo, and BP was measured 60 minutes after the first intranasal administration of placebo. Treatment period–Blood pressure was measured after 8 weeks of treatment with insulin (4×40 IU/d) or placebo. One hour before measurements, subjects received placebo spray. Data are expressed as mean ± SEM. Right column indicates the effects of treatments, which were all nonsignificant.

4. Discussion

In animals, infusion of insulin into brain-perfusing vessels induces an elevation of BP that distinctly exceeds that after infusion into peripheral vessels [35-37], suggesting that the increasing effect of insulin on BP may be mediated via central nervous mechanisms. It is as yet unclear if there is also an insulin-dependent contribution of the central nervous system to BP regulation in human beings. Using the intranasal route of insulin administration, which is known to convey drugs such as insulin from the nasal cavity to the cerebrospinal fluid compartment without absorption into the blood stream [22-29], we provide evidence that immediately increasing brain insulin levels elevates BP. However, the effect vanishes after prolonged treatment. Because long-term intranasal insulin administration has already yielded beneficial effects on memory processing [32] and on the regulation of body fat stores [33], the latter finding is of particular relevance because it demonstrates that long-term intranasal administration of insulin is not accompanied by an adverse hypertensive action.

Immediate intranasal insulin administration in human beings is followed by an increase in BP, as likewise seen after infusion of insulin to brain-perfusing vessels in animals [35-37]. For instance, in a previous animal study, the mean increase in BP after perfusion of brain vessels exceeded that after peripheral insulin infusion by approximately 17.00% ± 5.00% [35]. The increase in BP after perfusing brain vessels with insulin is not induced by a direct central nervous sympathoexcitatory action that has been observed after intracerebroventricular injection of insulin [47]. However, it is also unlikely that this increase reflects a peripheral action of insulin. Infusion of insulin into a peripheral vein induces a nitric oxide–mediated vasodilatation that induces a baroreflexive increase in sympathetic outflow to the muscle

vascular bed [40,48]. Such a peripheral effect that results in sympathoexcitation would be expected to induce hypertension only in subjects with an altered set point in BP regulation, in that primarily higher BP values are accepted prior to a reflexive suppression of sympathetic activity. However, in the present experiment, the effect of insulin appears to pertain directly to the set point of BP regulation because the increase in BP was not followed by a suppression of heart rate or MSNA. Both parameters remained entirely unchanged by the insulin treatment.

Apart from the sympathetic nervous system, the HPA axis is involved in the regulation of BP [49-52] and, via enhanced secretory activity, might contribute to the hypertensive impact of immediately administered intranasal insulin. This view seems plausible in light of studies demonstrating that intravenous infusion of insulin increases HPA secretory activity [53,54]. However, in the present experiments, immediate intranasal administration of insulin did not stimulate HPA secretory activity as indicated by unchanged plasma ACTH levels, excluding any contribution of HPA secretory activity to the immediate increase in BP after intranasal insulin.

It might be also argued that the increase in BP values seen during the immediate insulin experiment was induced by changes in plasma glucose levels. This view is primarily based on results of previous studies demonstrating a positive association between BP and plasma glucose levels [55,56]. However, statistical comparisons of plasma glucose levels between the insulin and placebo conditions did not result in significant differences, thereby excluding any contributions of plasma glucose levels to the hypertensive effect seen after the immediate intranasal treatment with insulin.

In contrast to its immediate effects, subchronic intranasal insulin administration did not induce a comparable increase in BP, suggesting that the mechanisms mediating insulin effects on long-term BP regulation differ from those involved in the immediate effects on BP regulation. This view fits well with studies indicating that long-term in contrast to immediate infusions of insulin into the cerebral circulation also did not cause a hypertensive shift of arterial pressure [35-39,57,58]. In combination with previous observations, our data point to a gradual activation of counterregulatory mechanisms during prolonged treatment with insulin. However, the mechanisms remain to be further characterized.

In summary, our results demonstrate that immediate intranasal administration of insulin enhances BP, thereby adding to previous findings showing similar increases in BP after immediate intravenous administration of insulin [40-42,48]. Furthermore, the present data indicate an essential contribution of central nervous system pathways to the effect of insulin on BP in human beings. Whereas rises in BP observed after intravenous administration of insulin have been assumed primarily to be caused by a direct activation of stress systems (ie, sympathetic nervous system and HPA axis) [39,59,60], the immediate rise in BP after intranasal administration of insulin points to central nervous mechanisms contributing to insulin-dependent BP regulation by a down-regulation of baroreflex sensitivity. However, this effect vanishes with prolonged intranasal administration of insulin. The absence of hypertensive effects after long-term intranasal administration of insulin is of considerable relevance for the possible clinical use of intranasal insulin in the treatment of disorders of memory and body weight regulation because it excludes a serious adverse effect of such therapies.

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