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Effect of intracerebroventricularly injected insulin on urinary sodium excretion by cerebroventricular streptozotocin-treated rats

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Abstract

Recent evidence suggests that insulin may influence many brain functions. It is known that intracerebroventricular (*icv*) injection of nondiabetogenic doses of streptozotocin (STZ) can damage insulin receptor signal transduction. In the present study, we examined the functional damage to the brain insulin receptors on central mechanisms regulating glomerular filtration rate and urinary sodium excretion, over four periods of 30 min, in response to 3 µl insulin or 0.15 NaCl (vehicle) injected *icv* in STZ-treated freely moving Wistar-Hannover rats (250-300 g). The *icv* cannula site was visually confirmed by 2% Evans blue infusion. Centrally administered insulin (42.0 ng/µl) increased the urinary output of sodium (from 855.6 ± 85.1 to 2055 ± 310.6 Δ%/min; N = 11) and potassium (from 460.4 ± 100 to 669 ± 60.8 Δ%/min; N = 11). The urinary sodium excretion response to *icv* insulin microinjection was markedly attenuated by previous central STZ (100 µg/3 µl) administration (from 628 ± 45.8 to 617 ± 87.6 Δ%/min; N = 5) or by *icv* injection of a dopamine antagonist, haloperidol (4 µg/3 µl) (from 498 ± 39.4 to 517 ± 73.2 Δ%/min; N = 5). Additionally, insulin-induced natriuresis occurred by increased post-proximal tubule sodium rejection, despite an unchanged glomerular filtration rate. Excluding the possibility of a direct action of STZ on central insulin receptor-carrying neurons, the current data suggest that the insulin-sensitive response may be processed through dopaminergic D1 receptors containing neuronal pathways.

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Chronic elevated plasma insulin levels and resistance to the hypoglycemic effect of insulin are associated with increased blood pressure in human and animal models of hypertension. This observation has led to the speculation that insulin may play a role in the development of increased blood pressure (1,2). On the other hand, the role of the CNS in the control of blood pressure and hydrosaline homeostasis has been demonstrated by several studies (3,4). Recently, increasing evidence has suggested not only that insulin

is vital to the brain but that the hormone may also exert an influence by modulating many brain functions, such as food intake regulation and cardiovascular function (1,2,5). Evidence for a selective insulin transport mechanism across the blood-brain barrier and its localization in specific brain regions supports a CNS regulatory function for insulin-related peptides (6-8).

We have recently demonstrated the involvement of insulin-induced insulin receptor and post-receptor protein phosphorylation in the action of insulin in rat hypothalamus (9). The peripheral action of insulin has been shown to reduce urinary sodium excretion, suggesting an attractive link between the renal effects of insulin, low urinary sodium excretion and the development and/or maintenance of arterial hypertension. We, as well as others (5,10,11), have shown that acute intracerebroventricular (*icv*) injection of insulin significantly decreased blood pressure, heart rate and renal sodium reabsorption, with corresponding reductions in renal sympathetic nerve activity in anesthetized rats. However, there is little information on the neural mechanisms mediating the effects of *icv* insulin administration on renal sodium handling by rats (10).

Functional damage to the neuronal insulin receptor signal transduction in the CNS comparable to type 2 diabetes mellitus has been established by *icv* injection of streptozotocin (STZ) in rats at levels below those producing diabetes (12). Also, a decrease in dopamine content and D1 receptor density has been demonstrated (13) in STZ-treated animals. Like cocaine, insulin or a nonapeptide C-terminus of the insulin ß-chain strongly inhibits dopamine uptake by the rat dopamine transporter stably expressed in Chinese hamster ovary cells (designated D8 cells). This inhibitory effect was also confirmed by experiments on striatal synaptosomes (14). Thus, we may hypothesize that CNS insulin and insulin-derived peptides are neuropeptides acting on neuronal insulin receptors and/or as neuropeptide precursors that may interact with the dopamine neuronal pathways to promote changes in renal sodium excretion.

In the present study, we examined the functional damage to the neuronal insulin receptor signal transduction on central mechanisms regulating urinary sodium excretion in *icv* STZ-treated rats. For this purpose, we assessed the effect of acute *icv* insulin administration on tubular sodium handling in unanesthetized, unrestrained rats.

Rats were randomly assigned to six groups: a) *icv* 0.15 M NaCl-injected rats (N = 11), b) *icv* 42.0 ng/µl insulin-injected rats (N = 11), c) STZ-treated rats (100 µg/3 µl) injected *icv* with 0.15 M NaCl (N = 5), or d) with insulin (N = 5), e) subcutaneously (*sc*) insulin-injected rats (N = 5), and f) *icv* 4 µg/3 µl haloperidol-treated rats (N = 5). The general guidelines established by the Brazilian College of Animal Experimentation (COBEA) were followed throughout the study.

Male Wistar-Hannover rats (250-300 g) were chronically instrumented with an *icv* guide cannula (4,15) and maintained under controlled temperature and lighting conditions in individual metabolic cages, with free access to tap water and standard laboratory rodent chow. Briefly, the animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight) and a stainless steel cannula was stereotaxically implanted into the lateral cerebral ventricle 6 days before the experiments, using previously reported techniques and pre-established coordinates (4,10): anteroposterior 0.2 mm from bregma; lateral 1.5 mm, and vertical 4.0 mm. The position of the cannula was visually confirmed by 2% Evans blue infusion through the *icv* cannula at the end of the experiment. STZ (100 µg/3 µl) was microinjected *icv* 7 days before administration of 42.0 ng/µl insulin.

Fourteen hours before the renal test, 60 µmol LiCl/100 g body weight was given by

gavage. After an overnight fast, each animal received a load of tap water by gavage (5% of body weight), followed by a second load of the same volume 1 h later. Thirty minutes after the second load (control period), vehicle (0.15 M NaCl) or insulin (100 U/ml, 206 mOsm/kg H_2O; Eli Lilly, São Paulo, SP, Brazil) was microinjected in the volume of 3 µl at the concentration of 42.0 ng/µl with a 10-µl Hamilton microsyringe and spontaneously voided urine was collected over four periods of 30 min into a graduated centrifuge tube.

At the end of the experiment, the animals were anesthetized with sodium pentobarbital, blood was drawn by cardiac puncture, and urine and plasma samples were obtained for analysis. Plasma and urine sodium, potassium and lithium concentrations were measured by flame photometry using a B262 Micronal Instrument (São Paulo, SP, Brazil), while creatinine concentrations and cerebrospinal fluid osmolarity were determined with a spectrophotometer (Micronal Instrument) and a wide-range osmometer (Advanced Inst. Inc., Norwood, MA, USA), respectively. Insulin levels were measured by radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA, USA) and plasma glucose concentration by an enzymatic method (Labtest, Campinas, SP, Brazil). Creatinine clearance (CCr) was used to estimate glomerular filtration rate and lithium clearance (CLi) was used to assess proximal tubule output (4,10). Fractional sodium excretion was calculated as CNa/CCr, where CNa is sodium clearance. The fractional proximal and post-proximal sodium excretion were calculated as CLi/CCr x 100 and CNa/CLi x 100, respectively. Renal parameter responses to *icv* insulin were calculated as the area under the curve versus time, with renal data reported as a percent of their baseline value during the 30-min control period preceding each experimental interval.

Statistical analysis of the data was performed using ANOVA for repeated measurements followed by the Bonferroni *post hoc* test to determine the extent of the differences. A P value ≤ 0.05 was considered to indicate statistical significance.

Figure 1 and Table 1 show the effects of *icv* and *sc* insulin or 0.15 M NaCl microinjection with or without previous STZ treatment on renal sodium and potassium handling, glycemia and insulinemia levels expressed as mean ± SEM per 100 g body weight. All rats survived and were clinically healthy up to the sixth day after the cannula was positioned into the lateral cerebral ven-

Table 1. Effect of intracerebroventricular (*icv*) and subcutaneous (*sc*) insulin (Ins, 42 ng/µl) or *icv* NaCl injection (control, 0.15 M) in sham-operated rats on cerebrospinal fluid (CSF) osmolarity, serum sodium, potassium and lithium levels, and insulinemia and glycemia compared to the administration of insulin (STZ + Ins, 42.0 ng/µl) in streptozotocin-treated rats.

Group	Sodium (mM)	Potassium (mM)	Lithium (µM)	Insulinemia (AUC)	Glycemia (AUC)	CSF osmolarity (mOsm/kg H_2O)
Control (*icv*, N = 11)	143 ± 2.2	4.1 ± 0.3	85 ± 13	14.91 ± 2.78	436 ± 21	306 ± 2.0
Ins (*icv*, N = 11)	145 ± 2.4	3.9 ± 0.4	60 ± 10	18.48 ± 2.02	298 ± 27*	301 ± 3.0
STZ + Ins (*icv*, N = 5)	143 ± 2.9	4.0 ± 0.7	140 ± 30	15.21 ± 3.34	387 ± 46	ND
Ins (*sc*, N = 5)	143 ± 3.5	3.5 ± 0.2	100 ± 10	13.2 ± 1.2	391 ± 37	ND

Data are reported as means ± SEM. AUC = area under the curve. ND = not determined. N = 5 for all measurements except insulinemia (Ins, *icv*, N = 6), glycemia (N = 7) and CSF osmolarity (N = 3).
*P ≤ 0.05 compared to respective control (ANOVA and Bonferroni *post hoc* test).

tricle. There were no significant differences in cerebrospinal fluid osmolarity, serum sodium, potassium or lithium levels (Table 1) between *icv* 0.15 M NaCl-injected rats and the other groups.

The urinary flow rates and the glomerular filtration rate estimated by CCr did not differ significantly among groups during the studies of renal tubule sodium handling (Figure 1). The *icv* microinjection of insulin (42.0 ng/µl) increased urinary fractional sodium excretion (from 855.6 ± 85.1 to 2055 ± 310.6 Δ%/min) and fractional potassium excretion in insulin-injected rats (from 460.4 ± 100 to 669 ± 60.8 Δ%/min). The urinary sodium excretion response to *icv* insulin injection was significantly attenuated by previous *icv* administration of 100 µg/3 µl STZ in rats (from 628 ± 45.8 to 617 ± 87.6 Δ%/min) (Figure 1). The enhanced fractional sodium and potassium excretions were accompanied by a significant increase in post-

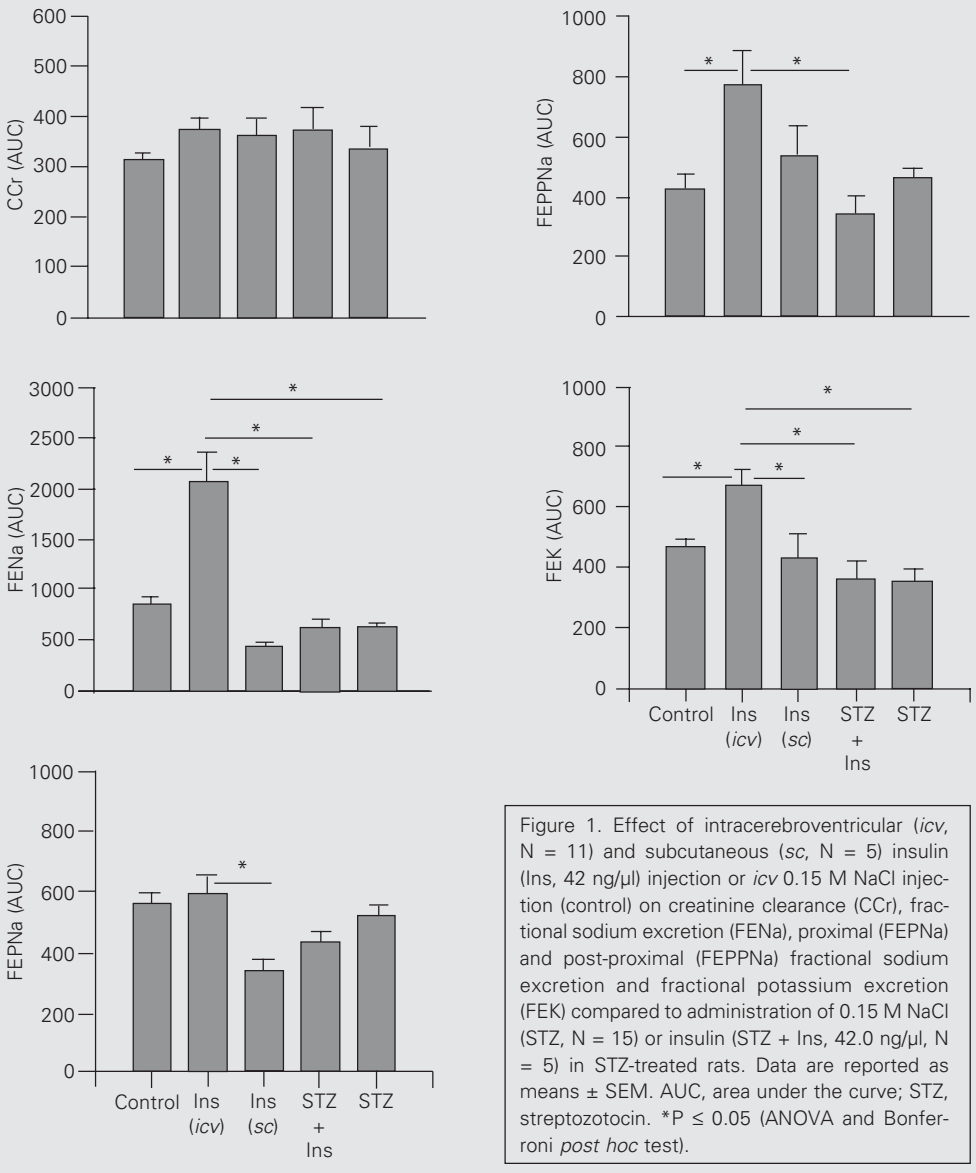

Figure 1. Effect of intracerebroventricular (*icv*, N = 11) and subcutaneous (*sc*, N = 5) insulin (Ins, 42 ng/µl) injection or *icv* 0.15 M NaCl injection (control) on creatinine clearance (CCr), fractional sodium excretion (FENa), proximal (FEPNa) and post-proximal (FEPPNa) fractional sodium excretion and fractional potassium excretion (FEK) compared to administration of 0.15 M NaCl (STZ, N = 15) or insulin (STZ + Ins, 42.0 ng/µl, N = 5) in STZ-treated rats. Data are reported as means ± SEM. AUC, area under the curve; STZ, streptozotocin. *P ≤ 0.05 (ANOVA and Bonferroni *post hoc* test).

proximal sodium rejection compared with the sham-operated *icv* 0.15 M NaCl-injected rats. This increase occurred despite an unchanged CCr and fractional proximal sodium excretion (Figure 1). The high fractional sodium excretion induced by *icv* insulin injection was blunted and significantly reduced by previous *icv* STZ administration ($P < 0.01$) (see Figure 1). This attenuated urinary ion excretion was associated with a significant decrease in proximal and post-proximal sodium rejection (Figure 1). Confirming previous studies, the renal natriuretic responses were not altered by *icv* 0.15 M NaCl administration. Likewise, CCr, natriuresis and kaliuresis were unaffected by insulin administered *sc* (Figure 1). Repeated *icv* microinjection of 42.0 ng/µl insulin caused reproducible decreases in glycemia levels ($P < 0.03$) (Table 1). On the other hand, insulinemia was not altered by *icv* insulin or 0.15 M NaCl microinjection (Table 1). Supporting the possibility that the action of insulin on the CNS may be modulated by central dopaminergic receptors, we demonstrated that the urinary sodium excretion response to *icv* insulin microinjection was markedly attenuated by 20-min previous *icv* injection of a dopamine antagonist, haloperidol (4 µg/3 µl; from 498 ± 39.4 to 517 ± 73.2 Δ%/min) accompanied by an unchanged glomerular filtration rate (CCr). There were no significant changes in kaliuresis in haloperidol + insulin-treated animals.

The fact that lesions of hypothalamic nuclei produce hyperinsulinemia and insulin resistance suggests a major role for the CNS in the regulation of insulin action and secretion. On the other hand, several lines of evidence indicate that hyperinsulinemia produces sympathetic activation in both humans and rats (5,15). In the current study, we demonstrated that centrally administered insulin produced substantial increases in the urinary output of sodium, lithium and potassium, and tested the hypothesis that the centrally insulin-induced renal ion excretion may, at least in part, be related to changes in CNS dopaminergic receptor integrity. In addition, we showed that insulin-induced natriuresis occurred by increasing post-proximal tubule sodium rejection despite an unchanged CCr (Figure 1) and was proportional to the sodium filtered load. Thus, the observed increase in renal fractional sodium and potassium excretions may be due to the inability of renal tubules to handle the electrolytes, with a consequent disruption in glomerulotubular balance.

It has been shown that insulin infused into the cerebroventricular space can reach neuronal loci after passing between ependymal cells or glial processes, to enter the interstices of the underlying cerebral neuropil (6-8). Injection of labeled insulin into the cerebral ventricles of rats produced heavy staining in regions closer to the third ventricle. *In vivo* and *in vitro* autoradiographic techniques have identified insulin-specific binding sites in the median eminence (8), the dorsomedial hypothalamus, the arcuate nucleus and the ventromedial hypothalamus (16). Although a physiological role of central insulin remains to be identified, insulin binding to axonal or synaptic receptors in the CNS influences hypothalamic norepinephrine release and peripheral autonomic function (11). Studies have shown that the injection of insulin into the periventricular area significantly reduces the efferent firing rate of peripheral sympathetic nerves and that this hypothalamic effect of insulin is abolished when neurons are destroyed by injection of kainic acid (11). It has also been demonstrated that *icv* injection of methylatropine suppresses the insulin responses to an oral glucose load in rats (17). We, as well as others (4,18), have shown that carbachol and norepinephrine injection into the septal area, anterior lateral hypothalamus, and subfornical organ as well as the anterior portion of the third ventricle induces a dose-related natriuresis accompanied by a lesser degree of kaliuresis (18).

All of these findings led us to hypothesize that the natriuresis observed in the present study may result from a significant and transient renal sympathetic inhibition or indirectly from a contribution of sympathetic and parasympathetic nervous system activation. In the present study, a possible indirect mechanism underlying the increase in renal sodium excretion includes insulin-induced changes in CNS glucose metabolism. Supporting such a mechanism, glucose deprivation in the CNS generated by *icv* injection of 2-deoxy-D-glucose increased peripheral autonomic nervous system activity in rats (19). However, an experiment using relatively large doses of *icv* insulin or cultured neurons labeled with radioactive 2-deoxy-D-glucose supported the traditional view that the brain is not responsive to insulin with respect to glucose uptake and metabolism (20). Furthermore, in a recent study, relatively large doses of *icv* insulin did not change peripheral neural activity, supporting our conclusion that the insulin effect in the present study was not mediated by glucose deprivation.

We showed that central insulin by itself decreased blood glucose levels, with no change in insulinemia. Intracerebroventricular application of low, nondiabetogenic doses (<500 μg/kg) of STZ is followed by alterations of the dopaminergic system in the rat striatum (13,14). In this brain region the dopamine content and dopaminergic D1 receptors were significantly decreased 7 days after *icv* administration of STZ compared to control (13,14). The findings of the present study suggest that dopaminergic receptors may, at least in part, modulate insulin signaling mechanisms associated with a rise of post-proximal sodium rejection (Figure 1), after central insulin injection. Since nondiabetogenic *icv* administration of STZ produces changes in striatal dopamine content and D1 receptor density, this system might be related to alterations of the brain insulin circuit, and in the present study in particular to the blunted urinary sodium excretion response in previously STZ-treated rats. This statement may also be supported by the attenuated urinary sodium excretion response to *icv* insulin microinjection after previous *icv* haloperidol administration shown in the present study.

However, other possibilities should be examined. First, a direct effect of STZ on insulin receptor-carrying neurons must be ruled out. Second, in view of the wide spectrum of STZ toxicity, other neurotransmitter systems or neuronal ensembles might also be compromised by STZ pretreatment. Recently, it has been shown that many brain-specific natriuretic factors are located in periventricular structures related to water and salt balance control (3,4,18), indicating a possible link between insulin and natriuresis. Since the mechanism of the STZ-attenuated natriuretic response in centrally insulin-injected rats is still unclear, investigations directed at finding insulin-sensitive pathways in periventricular areas may contribute to improving our understanding of the link between hypertension, sodium handling and diabetes.

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