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Effects of Pharmacological Lesion of Adrenergic Innervation of the Dorsal Vagal Nucleus on Pancreatic Insulin Secretion in Normal and Vagotomized Rats

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Summary

Previous morphological and physiological studies have suggested that the adrenergic innervation of the dorsal motor nucleus of the vagus nerve (dmnX) is involved in direct synaptic inhibition of parasympathetic preganglionic neurones of the vagus that control secretion of pancreatic insulin. We investigated the effects of bilateral 6-hydroxydopamine (6-OHDA) lesions of adrenergic innervation of the dmnX on pancreatic insulin secretion and glycaemia in normal and vagotomized rats. After two weeks the 6-OHDA lesions produced a marked increase in circulating insulin levels, but no change in glycaemia. Hyperinsulinaemia after adrenergic denervation of the dmnX was more pronounced when a glucose bolus was injected intraarterially. Bilateral subdiaphragmatic vagotomy reversed the observed hyperinsulinaemia. This targeted pharmacological lesion of the adrenergic innervation of dmnX thus causes hypersecretion by pancreatic B cells, an effect which requires an intact vagus nerve.

Key words

Dorsal motor nucleus of the vagus nerve – Adrenergic innervation – 6-OHDA lesion – Pancreatic insulin secretion – Vagotomy – Rats

Introduction

The dorsal vagal complex is involved in the central regulation of a number of visceral autonomic functions (Nauta 1972). This region of the dorsomedial medulla oblongata incorporates the nucleus of the tractus solitarius (NTS) and the area postrema, which are the major targets of vagal sensory information, and the dorsal motor nucleus of the vagus (dmnX), which provides the major cephalic, thoracic and upper abdominal viscera with their preganglionic vagal innervation. The dorsal motor nucleus of the vagus nerve provides preganglionic vagal innervation to the pancreas (Siaud *et al.* 1990). The dmnX has a major secretomotor role in the pancreas (Ionescu *et al.* 1983) and pancreatic insulin secretion is inhibited by bilateral vagotomy (Frohman *et al.* 1967).

Medullary adrenergic neurones have been assumed to have a role in the central regulation of visceral autonomic functions controlled by the vagus nerve (Howe *et al.* 1981). Large amounts of adrenaline and PNMT (phenylethanolamine-N-methyltransferase) – the final enzyme in adrenaline synthesis pathway – are present in both sensory and motor nuclei of the vagal complex of the dorsal medulla oblongata (Saavedra *et al.* 1974, van der Gugten *et al.* 1976, Koslow and Schlumpf 1981). Immunocytochemical techniques have identified a number of PNMT-containing perikarya in this region of the brainstem, in particular in the dorsomedial portions of the NTS, and numerous immunoreactive fibres in the dmnX (Siaud *et al.* 1989). These adrenergic fibres establish synaptic

connections with neurones innervating the pancreas. Measurements of plasma insulin levels indicate that visceral secretion is rapidly and conspicuously decreased by local infusion of adrenaline within the DVC (Siaud *et al.* 1990). These data strongly suggest that the adrenergic innervation of the dorsal medulla is involved in direct synaptic inhibition of the parasympathetic preganglionic neurones of the vagus that control secretion of pancreatic insulin (Siaud *et al.* 1989, 1990). The present paper describes a continuation of our previous analysis of central nervous control of insulin secretion. We have investigated the long-term effects of the adrenergic depletion induced by an intramedullary injection of 6-OHDA centered on the dmnX in normal and vagotomized rats on the basal levels of plasmatic insulin and glucose as well as on the glucose-stimulated levels of insulin.

Methods

A total of 39 control and experimental male Sprague Dawley rats, each weighing approximately 200 g, were used. All animals received a pelleted laboratory chow diet and had free access to tap water. Rats were housed individually in cages, in rooms at constant temperature (21±1 °C) and with a 12 h light-dark cycle. After one week of habituation, animals under deep equitestine anaesthesia (4 ml/kg) were placed in a stereotaxic device with the head in the nose down position. The dorsal cervical musculature was resected and the obex region of the dorsal medulla was exposed after removing the occipital skull plate. Intramedullary microinjections were given with a 1 μl Hamilton microsyringe fitted with a glass micropipette with an outside tip diameter of 40–50 μm. Both the stereotaxic coordinates of the injection site and volume of the injection were determined to impregnate the dnmX throughout its rostro-caudal extent as follows. The tip of the micropipette was positioned about 0.1 mm anterior, 0.4 mm lateral to the obex and 0.5 mm ventral to the brain surface.

The rats were divided into two groups. The control animals received a bilateral intramedullary injection of 0.15 M saline containing 1 mg/ml ascorbic acid solution and the experimental group, received 6-hydroxydopamine (6-OHDA, 10 μg in 0.5 μl solvent), a specific neurotoxin for catecholaminergic neurones. After one week, both the 6-OHDA- and the saline-injected groups were subdivided into two groups.

In the vagotomized rats both vagal trunks were exposed subdiaphragmatically, freed from surrounding connective tissue and cut. The completeness of vagotomy was verified under a binocular microscope. In the sham-operated animals the subdiaphragmatic vagal trunks were only exposed.

Thus, there were 4 groups: the NaCl-injected and sham-vagotomized control group (group 1), the 6-OHDA-injected and sham-vagotomized group (group 2), the NaCl-injected and vagotomized group (group 3) and the 6-OHDA-injected and vagotomized group (group 4).

Experiment 1: One week after vagotomy, half of the animals in each group were decapitated at 0900 h and blood samples were collected on EDTA. The samples were centrifuged at 4 °C and plasma vials were stored at −20 °C until the insulin and glucose assays.

Experiment 2: On the day of vagotomy, a polyethylene cannula was also implanted into the carotid artery of the other half of animals in each group, as previously described (Szafarczyk *et al.* 1980). Five days later, blood samples (for basal measurements) were collected from each animal, through this cannula 15 min and one minute before injection of 0.5 g/kg of glucose (10 % wt/vol) during 30 s into the carotid artery. Five and 15 min after the glucose injection, blood was withdrawn for measurements in the glucose-stimulated condition.

Plasma insulin and glucose determinations

Insulin was assayed by the method of Herbert *et al.* (1965) using an antibody supplied by Miles Laboratories (Paris, France). [^{125}I] insulin was obtained from international CIS (Gif/Yvette, France). The standard used was pure rat insulin (Novo, Copenhagen, Denmark) whose biological activity was 22.3 μU/ng. The intra- and inter-assay coefficients of variation were 9.7 % and 14.4 %, respectively, and the sensitivity was 0.2 ng/l. Blood glucose levels were measured by the glucose oxidase method (glucose enzymatic colour AO 2640, Biotrol).

Results

Experiment 1: The data on insulin, glucose and body temperature from the first experiment are presented in Fig. 1. Plasma insulin levels (Fig. 1A) were significantly higher in intramedullary 6-OHDA-injected rats (group 2: 5.2±0.9 mg/ml) than in the control group (group 1: 1.9±0.7 mg/ml). Bilateral subdiaphragmatic vagotomy in the sham-injected rats (group 3) caused a fall in plasma insulin (0.8±0.3 mg/ml) as compared with the control group 1 (1.9±0.7 mg/ml) and completely reversed the hyperinsulinaemia developed in the sham-operated group (2.33±1.0 mg/ml in group 4 vs 5.2±0.9 mg/ml in group 2). In contrast, the plasmatic glucose levels in groups 2, 3 and 4 were similar to control values (Fig. 1B) (group 1: 134±7; group 2: 145±9; group 3: 139±12; group 4: 156±15 mg/100 ml). The hyperinsulinaemia following intramedullary 6-OHDA injection and the decrease in plasma insulin of vagotomized rats were not accompanied by a corresponding decrease or increase in glycaemia, respectively.

Experiment 2: The second set of experiments compared the effects of intraperitoneal glucose injection on glucose (not illustrated) and insulin (Fig. 2) levels in the different experimental groups. Plasma glucose levels at the beginning of the experimental period were the same in all four groups of animals but the insulin levels were nearly twice as high in the 6-OHDA-treated animals (Fig. 2) than in the three other groups.

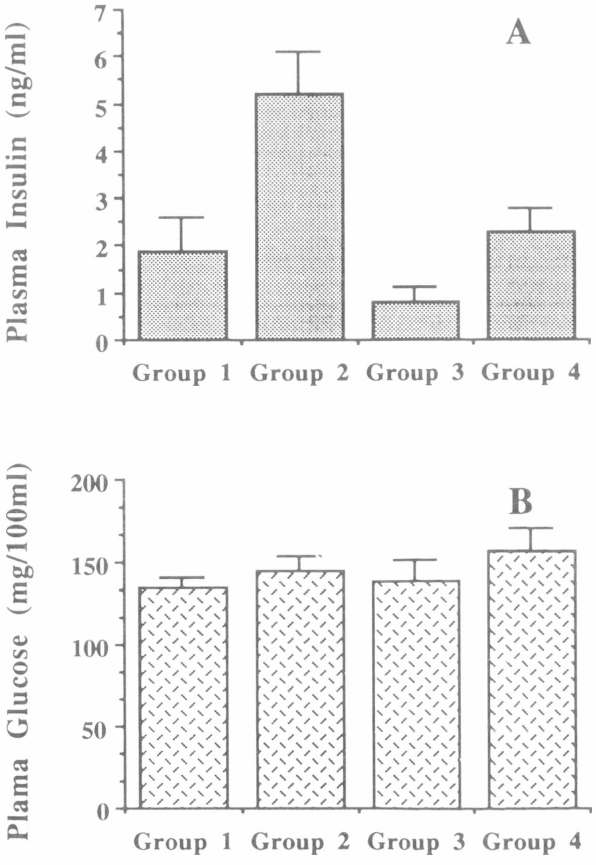

Fig. 1
The effects of 6-OHDA or vehicle injections into the nmdX on plasma levels of insulin (A) and glucose (B) in normal and vagotomized rats (n=6 in each group). Group 1: vehicle-injected and sham vagX, group 2: 6-OHDA-injected and sham vagX, group 3: vehicle-injected and vagX, group 4: 6-OHDA-injected and vagX. Values given are means±S.E.M. Statistical difference from group 1 values, $p<0.05$ (t-test). N=6.

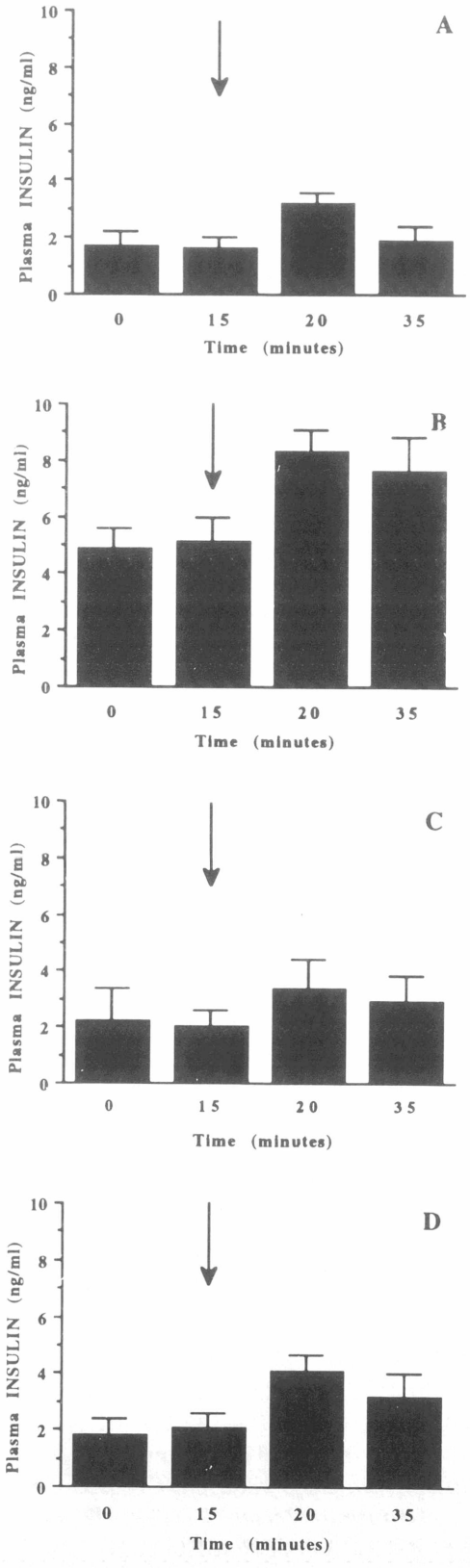

Fig. 2
Effects of injection of 6-OHDA or vehicle on plasma insulin levels of vagX and sham-operated rats. (A) group 1: vehicle-injected and sham vagX, N=6. (B) group 2: 6-OHDA-injected and sham vagX, N=5. (C) group 3: vehicle-injected and vagX, N=5. (D) group 4: 6-OHDA injected and vagX, N=5. A bolus injection of glucose was given at t=15 min (arrows). Data are means±S.E.M.

After the glucose injection, plasma insulin levels of the 6-OHDA-injected animals (group 2) increased from 5.1±0.9 (n=5) to 8.2±0.8 ng/ml (n=5) (Fig. 2A) and in the sham-operated rats (group 1) the increase was from 1.6±0.4 (n=6) to 3.3±0.5 ng/ml (n=6) (Fig. 2B). These peak values were observed 5 min after the injection of glucose. Twenty minutes after the injection, the insulin concentration had declined by 20 %. The change in glucose levels was similar in both 6-OHDA- and NaCl-injected animals.

In the previously vagotomized rats, the plasma insulin response pattern of 6-OHDA- and NaCl-injected animals (groups 3 and 4) to glucose bolus injections were similar (Figs 2C-D). The insulin levels of these two groups rose from 1.6±0.6 (n=5) to 4.1±0.6 ng/ml (n=5), and from 2.0±0.6 (n=5) to 3.8±1.0 ng/ml (n=5), respectively. In addition, there were no changes in comparison to the control group 1 (Fig. 2A), even though the concentration of insulin rose.

In vagotomized rats, the effects of the glucose injection, of 6-OHDA (group 3) or NaCl (group 4) intramedullary injection on plasma glucose were often similar to those seen in sham-vagotomized rats (groups 1 and 2).

Discussion

This study demonstrates that specific 6-OHDA lesions cause an increase in insulin secretion which is more pronounced in the presence of an exogenous glucose challenge. This confirms and extends a previous finding which showed that a fall of insulin secretion occurs after acute infusion of adrenaline into the dmnX (Siaud *et al.* 1990). Furthermore, the small but significant fall in plasma insulin levels following vagotomy indicates that a part of the basal insulin secretion is under vagal influence. This is in agreement with Frohman *et al.* (1967) who demonstrated the chronic stimulatory influence of the vagus nerve on insulin secretion in the dog by studies involving both vagotomy and vagal stimulation. Our study thus strongly suggests neurally-mediated hyperinsulinaemia as a consequence of 6-OHDA lesion although this is not proven directly. In particular, the involvement of a humoral factor is not ruled out by the experimental approach used. Indeed, the existence of an insulinogenic hypothalamic factor has been suggested (Helman *et al.* 1982). The catecholaminergic innervation of the hypothalamus which originates from A2-C2 cell groups may be partially altered by the spreading of 6-OHDA over the NTS, causing perturbation of the secretion of the latter. However, the cessation of hyperinsulinaemia after vagotomy in our experiment is consistent with the 6-OHDA lesion-induced hyperinsulinaemia being mediated by the vagus nerve. There are thus parallels between our findings and those of Berthoud and Jeanrenaud (1979) in studies of the suppression of hyperinsulinaemia induced by ventromedial hypothalamic lesions after vagotomy. Their results suggest that integrity of the nervous link constituted by synaptic interrelations between adrenergic neurones and vagal preganglionic motoneurones is necessary for regulation of pancreatic insulin secretion (Inoue *et al.* 1983, Rohner-Jeanrenaud *et al.* 1983).

The absence of changes in blood glucose secretion following 6-OHDA injections or vagotomy appears to indicate a compensatory mechanism controlling blood glucose. It is possible that the vagus nerve may influence glucagon in a manner similar to that of insulin or there may be concomitant sympathetic stimulation.

Adrenergic innervation of the dmnX thus appears to exert strong inhibitory control on pancreatic insulin secretion. It is possible to stipulate that the regulation of this visceral function by the central nervous system at least partially involves adrenergic medullary pathways.

The results of this study support the hypothesis that A2-C2 cell groups and particularly the adrenergic innervation of the dmnX are involved in enhancing body weight both under pathological (such as the preobesity syndrome) and physiological conditions (for example, the prehibernating state).

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