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Insulin and glucagon secretion by ganglionic nicotinic activation in adrenalectomized mice

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Abstract

The pancreatic islets are innervated by nerves emanating from intra- and extrapancreatic ganglia. However, the effects of ganglionic activation on insulin and glucagon release in vivo have not been established. We therefore investigated the effects of pharmacological ganglionic activation by the nicotinic agonists DMPP (1,1-dimethyl-4-phenylpiperazinium iodide) and nicotine on insulin and glucagon release in sham-operated and adrenalectomized mice. In sham-operated animals, DMPP (0.5 or 1.6 μmol/kg, i.v.) or nicotine (0.075 or 0.75 μmol/kg, i.v.), did not affect plasma insulin levels, but markedly increased plasma glucagon levels ($P < 0.05$). In contrast, after adrenalectomy or α_2-adrenoceptor blockade by yohimbine (3.6 μmol/kg), nicotinic activation markedly increased plasma insulin levels ($P < 0.05$), whereas the glucagon response to nicotinic activation was inhibited under these conditions ($P < 0.05$). We conclude that pharmacological ganglionic nicotinic activation in mice stimulates insulin and glucagon secretion. The insulinotropic effect is, however, counteracted by a concomitant adrenal activation through an α_2-adrenoceptor-mediated mechanism. © 1998 Elsevier Science B.V.

Keywords: Islet; Pancreatic nerve; Ganglia; Insulin; Glucagon

1. Introduction

The islets of Langerhans are innervated by cholinergic, adrenergic and peptidergic nerve fibres all of which participate in the regulation of insulin and glucagon secretion (Honjin, 1956; Miller, 1981; Ahrén et al., 1986; Sundler and Böttcher, 1991; Yamaguchi, 1992). Thus, pancreatic parasympathetic nerves stimulate insulin and glucagon secretion, whereas pancreatic sympathetic nerves inhibit insulin secretion and stimulate glucagon secretion (Miller, 1981; Ahrén et al., 1986, for reviews).

The nerve fibres innervating the islets of Langerhans originate from ganglia either within or outside the pancreas. Thereby, the islet parasympathetic nerve fibres emanate from intrapancreatic ganglia which are under the influence of the vagal nerve and the islet sympathetic nerve fibres emanate mainly from the ganglion coeliacum (Honjin, 1956; Miller, 1981). The physiological importance of the autonomic ganglia for islet function is, however, far form established. Previously, pharmacological ganglionic activation of nicotinic receptors by nicotine in vitro, has been demonstrated to stimulate insulin release in the perfused dog and rat pancreas (Chapal and Loubatieres-Mariani, 1978; Stagner and Samols, 1985, 1986), whereas nicotine has been shown to be without effect on insulin release from isolated rat islets of Langerhans (Ejiri et al., 1990). Little is, however, known on the effects of pharmacological ganglionic activation on insulin and glucagon secretion under in vivo conditions. Such knowledge would provide an insight into the integrated neural influences on islet hormone secretion, since both parasympathetic and sympathetic ganglia would be activated by nicotinic activation in the in vivo situation when the extrinsic innervation of the pancreas is intact.

The present study was therefore designed to investigate the effect of pharmacological ganglionic nicotinic activation on insulin and glucagon secretion under in vivo conditions in mice. We used two nicotinic ganglionic agonists, nicotine and DMPP (Gyermek, 1980), which were injected intravenously to conscious mice. Since nicotinic activation in vivo releases adrenal catecholamines (Gaspo et al., 1994), which are known to inhibit insulin

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release (Mukherjee et al., 1985; Debuyser et al., 1991; Karlsson et al., 1997) and to stimulate glucagon release (Iversen, 1973; Skoglund et al., 1987), experiments were also undertaken in adrenalectomized animals or in animals pre-treated with the α_2-adrenoceptor antagonist yohimbine, in order to discriminate between neural ganglionic, and adrenal effects of nicotinic activation.

2. Materials and methods

2.1. Animals

Female mice of the NMRI strain (Bolmholdtgaard Breeding and Research Centre, Ry, Denmark) weighing 25–35 g were used throughout the study. The animals were fed a standard pellet diet and tap water ad libitum. The study was approved by the Ethic Committee for Animal Research of Lund University.

2.2. Adrenalectomy

The adrenals were removed by the lumbar approach under ether anaesthesia as described previously (Karlsson and Ahrén, 1991). Control animals were sham-operated, i.e. a lumbar incision was made, the adrenals were visualised and the incision was closed by metallic clips as in animals subjected to adrenalectomy. The animals were used for the experiments at 48 h after surgery. Adrenalectomized animals were offered saline to drink ad libitum and were maintained on the usual diet.

2.3. Experiments

Sham-operated or adrenalectomized mice were injected intravenously into a tail vein with either DMPP (1,1-dimethyl-4-phenylpiperazinium iodide; Sigma Chemical Co., St Louis, MO) or nicotine ([−]nicotine di[+]tartrate salt; Sigma). DMPP was used at the dose levels of 0.5 and 1.6 μmol/kg. Within this range of dose levels, DMPP has previously been used for in vivo studies in the dog and shown to increase the plasma catecholamine levels (Gaspo et al., 1994). Nicotine was used at the low dose levels of 0.075 and 0.75 μmol/kg. These dose levels are approximately ten times lower than those previously shown to induce clonic seizures in mice (Collins et al., 1988) and nicotine within this range of dose levels has previously been used for in vivo studies in the mouse (Lundquist, 1982). Controls of both adrenalectomized and sham-operated animals were injected intravenously with 0.9% (w/v) NaCl. The volume load was 10 μl/g body weight. At 2 min after the intravenous injection, blood (250 μl) was sampled from the retrobulbar venous plexus of conscious mice as previously described (Rerup and Lundquist, 1966). The short time period (2 min) between injection of the agonists and blood sampling was selected in order to optimise conditions for studying the secretion of the islet hormones and to avoid secondary effects on the secretion of insulin and glucagon by changes in the plasma glucose levels. Furthermore, previous studies from our laboratory have demonstrated that the peak increase in plasma insulin or glucagon after an intravenous injection of carbachol occurs after two min in the mouse (Ahrén and Lundquist, 1981, 1986). Yohimbine (3.6 μmol/kg; Serva, Heidelberg, Germany) was given intraperitoneally at 10 min before an intravenous injection of either nicotine or 0.9% (w/v) NaCl as described above. Controls were given 0.9% (w/v) NaCl intraperitoneally. At this dose level yohimbine has previously been demonstrated to reverse the inhibitory effect of clonidine on glucose-stimulated insulin secretion in mice (Skoglund et al., 1986). After sampling, the blood was immediately stored on ice, plasma was separated by centrifugation and stored at −20°C until assayed for its content of insulin, glucagon and glucose.

2.4. Determinations of insulin, glucagon and glucose

Insulin was determined by radioimmunoassay using a guinea-pig anti-rat insulin antibody, ^{125}I-labelled human insulin as tracer and rat insulin as standard (Linco Res., St. Charles, MO). The separation of free and bound radioactivity was performed by the double antibody technique using a goat anti-guinea-pig IgG antibody (Linco). Plasma glucagon levels were determined by radioimmunoassay using an antibody specific for pancreatic glucagon, ^{125}I-labelled glucagon and glucagon as standard (Milab AB, Malmö, Sweden). In the yohimbine experiments plasma glucagon was determined by the use of a glucagon antibody, ^{125}I-glucagon and glucagon standard from Linco. Plasma glucose levels were determined with the glucose oxidase method.

2.5. Statistics

Data are presented as means ± S.E.M. One way analysis of variance (ANOVA) followed by Student–Newman–Keuls test or Students *t*-test for unpaired observations were used for statistical evaluation. A *P*-value of $P < 0.05$ was considered significant.

3. Results

3.1. Plasma insulin

Intravenous injection of the ganglionic nicotinic receptor agonists, either DMPP (0.5 or 1.6 μmol/kg) or nicotine (0.075 or 0.75 μmol/kg) did not significantly affect the plasma insulin levels in sham-operated control animals (Fig. 1a and Fig. 2a). In contrast, in adrenalectomized mice DMPP, at the highest dose level (1.6 μmol/kg), increased the plasma insulin levels (730 ± 226 pM compared to 180 ± 32 pM in NaCl-injected controls; $P < 0.05$; Fig. 1a). Similarly, in adrenalectomized animals, nicotine (0.75

μmol/kg) increased plasma insulin levels (622 ± 158 pM compared to 228 ± 42 pM in NaCl-injected controls; $P < 0.05$; Fig. 2a). Furthermore, in mice pre-treated with the α_2-adrenoceptor antagonist yohimbine (3.6 μmol/kg), nicotine (0.75 μmol/kg) increased plasma insulin levels to 884 ± 79 pM compared to 403 ± 89 pM in controls ($P < 0.05$; Fig. 3a). Thus, whereas nicotinic receptor activation does not affect plasma insulin levels under normal conditions, it stimulates insulin secretion in adrenalectomized animals as well as during α_2-adrenoceptor blockade.

3.2. Plasma glucagon

In sham-operated control animals, DMPP (0.5 or 1.6 μmol/kg) as well as nicotine (0.075 or 0.75 μmol/kg) dose-dependently increased plasma glucagon levels ($P < 0.05$; Fig. 1b and Fig. 2b). Also in adrenalectomized animals, both DMPP and nicotine, at the higher, but not at the lower dose levels, increased the plasma glucagon levels ($P < 0.05$; Fig. 1b and Fig. 2b). However, the glucagon responses at these higher dose levels of the agonists were

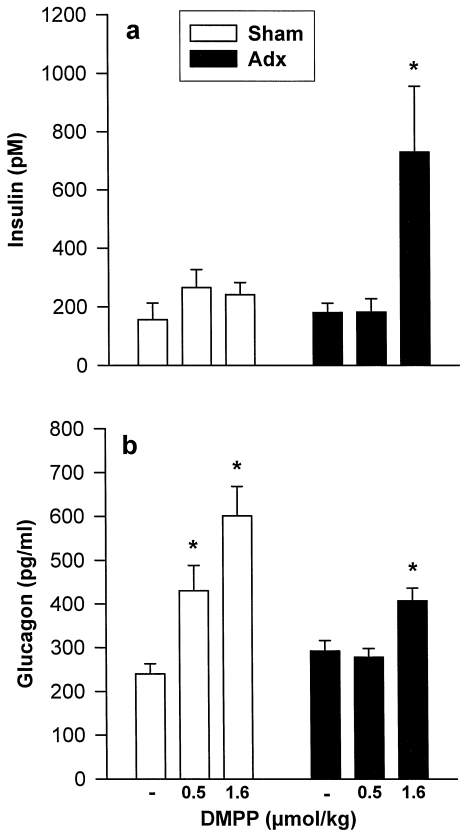

Fig. 1. Plasma insulin (a) and glucagon (b) levels at 2 min after an intravenous injection of DMPP (0.5 or 1.6 μmol/kg) to adrenalectomized (adx) or sham-operated (sham) mice. Means ± S.E.M. are shown. There were 7–8 animals in each group. Asterisk indicates probability level of random difference of at least $P < 0.05$ between DMPP-injected animals and respective NaCl-injected controls as determined by analysis of variance followed by Student–Newman–Keuls test.

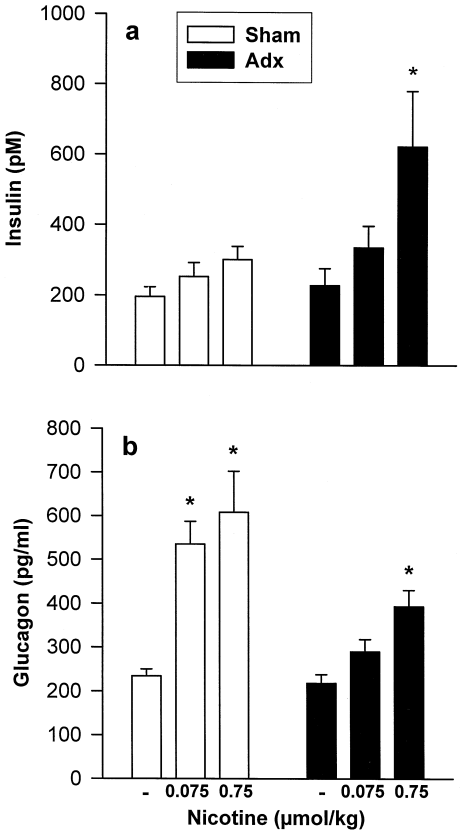

Fig. 2. Plasma insulin (a) and glucagon (b) levels at 2 min after an intravenous injection of nicotine (0.075 or 0.75 μmol/kg) to adrenalectomized (adx) or sham-operated (sham) mice. Means ± S.E.M. are shown. There were 7–8 animals in each group. Asterisks indicate probability level of random difference of at least $P < 0.05$ between nicotine-injected animals and respective NaCl-injected controls as determined by analysis of variance followed by Student–Newman–Keuls test.

hampered compared to in sham-operated controls. Thus, the glucagon response to DMPP (1.6 μmol/kg) was impaired by 68% ($P < 0.05$) and the glucagon response to nicotine (0.75 μmol/kg) was impaired by 53% by adrenalectomy ($P < 0.05$). In mice pre-treated with the α_2-adrenoceptor antagonist yohimbine (3.6 μmol/kg) the glucagon response to nicotine (0.75 μmol/kg) was hampered by 81% compared to NaCl pre-treated controls ($P < 0.05$; Fig. 3b). These results show that nicotinic agonism stimulates glucagon secretion through mainly an α_2-adrenoceptor mediated mechanism involving both adrenal activation and pancreatic adrenergic nerves.

3.3. Plasma glucose

Plasma glucose levels were lower in NaCl-injected adrenalectomized animals compared to in sham-operated animals (8.5 ± 0.3 versus 10.5 ± 0.3 mM; $P < 0.001$, n = 14–15), whereas yohimbine did not significantly affect plasma glucose levels (10.1 ± 0.2 in controls versus 9.1 ± 0.5 in yohimbine-treated mice, $n = 8$, n.s.). Nicotinic agonism did not affect plasma glucose levels in adrenalec-

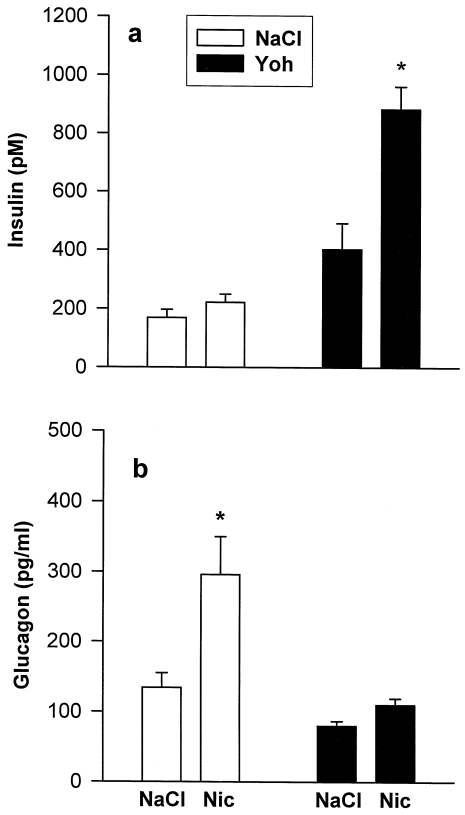

Fig. 3. Plasma insulin (a) and glucagon (b) levels at 2 min after an intravenous injection of nicotine (Nic; 0.75 μmol/kg) or NaCl to mice treated intraperitoneally with yohimbine (Yoh; 3.6 μmol/kg) or 0.9% (w/v) NaCl. Means ± S.E.M. are shown. There were 7–8 animals in each group. Asterisks indicate probability level of random difference of at least $P < 0.05$ between nicotine-injected animals and respective NaCl-injected control as determined by analysis of variance followed by Student–Newman–Keuls test.

tomized or yohimbine-treated animals, whereas in control animals, DMPP (1.6 μmol/kg; 12.1 ± 0.3 versus 10.1 ± 0.3 mM, $P < 0.05$, $n = 7–8$) and nicotine (0.75 μmol/kg; 11.3 ± 0.3 versus 10.4 ± 0.2 mM; $P < 0.05$: $n = 14–15$) slightly elevated the plasma glucose levels. Thus, since plasma glucose levels were not affected by nicotinic activation in adrenalectomized or yohimbine-treated animals, the increase in plasma insulin levels induced by nicotinic agonism in these animals is not secondary to changes in plasma glucose levels.

4. Discussion

The main finding of the present study is that ganglionic nicotinic activation by DMPP or nicotine under in vivo conditions in adrenalectomized mice leads to stimulation of both insulin and glucagon release. This demonstrates that the nerve fibres innervating the islets of Langerhans are modulated by ganglionic nicotinic activation and emphasises that the islet nerve fibres are involved in the regulation of the secretion of insulin and glucagon.

Our finding that plasma insulin levels were not affected by either of the nicotinic agonists DMPP or nicotine in sham-operated mice is in accordance with a previous study in normal mice demonstrating no effect on plasma insulin levels by nicotine (Lundquist, 1982). However, nicotinic activation in vivo is known to release adrenal catecholamines (Gaspo et al., 1994) and the catecholamines are well-known inhibitors of insulin release in the mouse (Mukherjee et al., 1985; Debuyser et al., 1991; Karlsson et al., 1997). Therefore, the fact that nicotinic activation was found to induce a marked insulin response after both adrenalectomy and during α_2-adrenoceptor blockade demonstrates that adrenal activation by nicotinic receptor agonism counteracts a stimulatory influence exerted by nicotinic activation of insulin secretion.

Previously, nicotine has been demonstrated not to affect insulin release from isolated rat islets or to affect the electrical activity of isolated mouse beta-cells (Santos and Rojas, 1989; Ejiri et al., 1990) demonstrating that the beta-cell itself is not equipped with nicotinic receptors. Furthermore, in the isolated perfused pancreas, with intact pancreatic ganglia, nicotine has previously been demonstrated to stimulate the release of insulin (Chapal and Loubatieres-Mariani, 1978; Stagner and Samols, 1985, 1986). Therefore, we interpret our finding of elevated plasma insulin levels induced by DMPP or nicotine in adrenalectomized or yohimbine-treated animals, to reflect increased insulin secretion in response to activation of postganglionic pancreatic nerves to the islets of Langerhans. Furthermore, our finding that nicotinic receptor activation did not affect plasma glucose levels in the adrenalectomized or yohimbine-treated animals shows that the insulin secretory response to nicotinic agonism in these animals is not secondary to changes in the plasma glucose levels.

The exact nature of the postganglionic nerves responsible for the insulinotropic response to nicotinic activation under in vivo conditions is at present not known. Previously, baseline insulin secretion from the isolated perfused dog pancreas in vitro has been shown to be regulated by intrapancreatic ganglia in a complex manner, comprising both cholinergic, adrenergic and peptidergic neural mechanisms (Stagner and Samols, 1986). Furthermore, nicotine-stimulated insulin secretion from the perfused rat pancreas in vitro has previously been demonstrated to be partially inhibited by atropine, suggesting the involvement of postganglionic cholinergic nerves in vitro (Chapal and Loubatieres-Mariani, 1978). However, the exact mechanisms involved in the insulinotropic response to nicotinic activation under in vivo conditions remains to be established, taking into account the cholinergic, adrenergic and peptidergic innervation of both the islets and the intrapancreatic ganglia.

In the present study, plasma glucagon levels were markedly increased by both DMPP and nicotine in sham-operated animals. This increase in plasma glucagon levels

was hampered by adrenalectomy and virtually abolished by α_2-adrenoceptor blockade. Since catecholamines stimulate glucagon release (Iversen, 1973; Skoglund et al., 1987) these findings demonstrate that adrenal catecholamines, to a major extent, and pancreatic adrenergic nerves, to a lesser extent, contribute to the glucagon response induced by nicotinic agonism. Thus, only a minor, if any, part of the glucagon response to nicotinic activation seems to involve non-α_2-adrenergic neural mechanisms which is in contrast to the insulin response to nicotinic activation. Hence, both postganglionic nerves and adrenal catecholamines contribute to the increased glucagon secretion observed after nicotinic receptor activation. However, it can not be excluded that also the hyperinsulinemia induced by nicotinic receptor activation in adrenalectomized and yohimbine-treated mice also might contribute to the hampered glucagon response to nicotinic activation during these conditions, since insulin is known to inhibit glucagon release (Samols et al., 1986).

In conclusion, under in vivo conditions in mice, nicotinic activation exerts both stimulatory and α_2-adrenoceptor-mediated inhibitory influences on insulin secretion. The stimulatory effect involves activation of postganglionic pancreatic nerves, whereas the inhibitory effect is exerted mainly by adrenal catecholamines. Furthermore, nicotinic activation stimulates glucagon secretion, which is mediated by both activation of postganglionic pancreatic nerves and the adrenals.

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References

Ahrén, B., Lundquist, I., 1981. Effects of selective and non-selective β-adrenergic agents on insulin secretion in vivo. Eur. J. Pharmacol. 71, 93–104.

Ahrén, B., Lundquist, I., 1986. Secretin potentiates cholinergically induced glucagon secretion in the mouse. Acta Physiol. Scand. 128, 575–578.

Ahrén, B., Taborsky, G.J. Jr., Porte, D., 1986. Neuropeptidergic versus cholinergic and adrenergic regulation of islet hormone secretion. Diabetologia 29, 827–836.

Chapal, J., Loubatieres-Mariani, M.M., 1978. Effects de la nicotine sur la secretion d'insuline du pancreas isole et perfuse de rat. C. R. Seances Soc. Biol. Fil. 172, 156–160.

Collins, A.J., Miner, L.L., Marks, M.J., 1988. Genetic influences on acute responses to nicotine and nicotine tolerance in the mouse. Pharmacol. Biochem. Behav. 30, 269–278.

Debuyser, A., Drews, G., Henquin, J.-C., 1991. Adrenaline inhibition of insulin release: Role of cyclic AMP. Mol. Cell. Endocrinol. 78, 179–186.

Ejiri, E., Taniguchi, H., Ishihara, K., Hara, Y., Baba, S., 1990. Possible involvement of nicotinic receptor in insulin release from isolated rat islets. Diabet. Res. Clin. Pract. 8, 193–199.

Gaspo, R., Yamaguchi, N., de Champlain, J., 1994. Nifedipine inhibits adrenal but not circulating catecholamine response to nicotinic stimulation in dogs. Am. J. Physiol. 267, R1545–1551.

Gyermek, L., 1980. Methods for the examination of ganglion-blocking activity. In: Kharkevich, D.A. (Ed.), Pharmacology of Ganglionic Transmission, Handbook of Pharmacology, vol. 53. Springer, New York, pp. 63–121.

Honjin, R., 1956. The innervation of the pancreas of the mouse, with special reference to the structure of the peripheral extension of the vegetative nervous system. J. Comp. Neurol. 104, 331–371.

Iversen, J., 1973. Adrenergic receptors and the secretion of glucagon and insulin from the isolated, perfused canine pancreas. J. Clin. Invest. 52, 2102–2116.

Karlsson, S., Ahrén, B., 1991. Insulin and glucagon secretion in swimming mice: Effects of adrenalectomy and chemical sympathectomy. J. Auton. Nerv. Syst. 32, 183–189.

Karlsson, S., Myrsén, U., Nieuwenhuizen, A., Sundler, F., Ahrén, B., 1997. Presynaptic sympathetic mechanism in the insulinostatic effect of epinephrine in mouse pancreatic islets. Am. J. Physiol. 272, R1371–R1378.

Lundquist, I., 1982. Cholinergic muscarinic effects on insulin release in mice. Pharmacology 25, 338–347.

Miller, R.E., 1981. Pancreatic neuroendocrinology: Peripheral neural mechanisms in the regulation of the islets of Langerhans. Endocr. Rev. 2, 471–494.

Mukherjee, B., Chatterjee, A.K., Bhatia, G.S., Mukherjee, S.K., 1985. Effect of epinephrine and norepinephrine on immuno-reactive insulin secretion from isolated islets of Langerhans. Biochem. Pharmacol. 34, 985–987.

Rerup, C., Lundquist, I., 1966. Blood glucose level in mice. 1. Evaluation of a new technique of multiple serial sampling. Acta Endocrinol. 52, 357–367.

Samols, E., Bonner-Weir, S., Weir, G., 1986. Intra-islet insulin–glucagon–somatostatin relationships. Clin. Endocrinol. Metab. 15, 33–58.

Santos, R.M., Rojas, E., 1989. Muscarinic receptor modulation of glucose-induced electrical activity in mouse pancreatic B-cells. FEBS Lett. 249, 411–417.

Skoglund, G., Lundquist, I., Ahrén, B., 1986. Effects of α_1- and α_2-adrenoceptor stimulation and blockade on plasma insulin levels in the mouse. Pancreas 1, 415–420.

Skoglund, G., Lundquist, I., Ahrén, B., 1987. α_1- and α_2-adrenoceptor activation increases plasma glucagon levels in the mouse. Eur. J. Pharmacol. 143, 83–88.

Stagner, J.I., Samols, E., 1985. Role of intrapancreatic ganglia in regulation of periodic insular secretions. Am. J. Physiol. 248, E522–E530.

Stagner, J.I., Samols, E., 1986. Modulation of insulin secretion by pancreatic ganglionic nicotinic receptors. Diabetes 35, 849–854.

Sundler, F., Böttcher, G., 1991. Islet innervation, with special reference to neuropeptides. In: Samols, E. (Ed.), The Endocrine Pancreas. Raven, New York, pp. 29–52.

Yamaguchi, N., 1992. Sympathoadrenal system in neuroendocrine control of glucose: Mechanisms involved in the liver, pancreas, and adrenal gland under hemorrhagic and hypoglycemic stress. Can. J. Physiol. 21, 648–653.