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Minireview

Galanin and the endocrine pancreas

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Galanin is a 29 amino acid peptide, initially isolated from the porcine small intestine. The peptide has been shown to occur in intrapancreatic nerves in close association to the islets. Its effects on islet hormone secretion and its possible mechanisms behind these effects are reviewed. Galanin has been shown to inhibit basal and stimulated insulin secretion both in vivo and in vitro under a variety of experimental conditions. The peptide has also been shown to inhibit somatostatin secretion and the secretion of pancreatic polypeptide (PP). With regard to glucagon secretion, however, results in the literature are not consistent since both stimulatory and inhibitory effects have been reported. A direct interaction with the pancreatic β-cells has been proposed behind its inhibitory action on insulin secretion, since galanin inhibits insulin secretion from isolated β-cells from obese, hyperglycaemic, mice. Galanin has thereby also been shown to induce repolarization and to reduce the free Ca^{2+} concentration, $[Ca^{2+}]_i$. The reduction in $[Ca^{2+}]_i$ is probably not due to a direct interference with the voltage-activated Ca^{2+} channels, since there is no effect of galanin when these channels are opened by depolarization induced by high concentrations of K^+. Instead, preliminary studies indicate that galanin activates the K^+ channels that are regulated by ATP, in turn inducing a repolarization-induced reduction in $[Ca^{2+}]_i$ resulting in reduced insulin secretion. However, the possibility that galanin inhibits the insulin secretory mechanism at a step distal to the regulation of cytoplasmic free Ca^{2+} concentration should not be overlooked.

Galanin; Insulin secretion; Glucagon secretion; Somatostatin secretion; Pancreatic polypeptide secretion; Ca^{2+}; (Pancreas, Islets of Langerhans)

1. INTRODUCTION

Galanin is a 29 amino acid peptide, initially isolated from the porcine small intestine [1]. The peptide is widely distributed within the body and exerts a variety of different effects [2]. In 1986, immunocytochemistry revealed that galanin occurs in intrapancreatic nerves in the dog [3]. The nerves were observed in the endocrine as well as in the exocrine portion of the pancreas and around blood vessels. However, the strongest fluorescence was consistently observed in close association with nerves innervating the pancreatic islets [3]. This finding corroborated an earlier observation of galanin-like immunoreactivity in the rat and pig pancreas, as determined by radioimmunoassay [4]. The localization of galanin to intrapancreatic nerves stimulated a series of new investigations concerning its effects on islet function and galanin is now included in the neuropeptidergic concept in the regulation of islet hormone secretion [5]. From the results obtained so far, it is clearly established that galanin has a major influence on islet hormone secretion under a variety of experimental conditions. Although the exact mechanisms mediating such an influence are poorly understood, recent studies have focussed the interest on the possibility of direct interference of galanin with basic biochemical, cell biological, and electrophysiological processes [6].

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2. BASAL PLASMA GLUCOSE AND INSULIN LEVELS

The first indication of an effect of galanin on the endocrine pancreas was the demonstration of a hyperglycaemic action of the peptide in dogs [1]. After 60 min of intravenous infusion of galanin, at a rate of 10 pmol/kg per min, plasma glucose levels increased from 107 ± 4 to 134 ± 9 mg/dl [1]. McDonald et al. [7] also demonstrated that 10 pmol/kg per min of galanin evoked a maximal elevation of basal plasma glucose levels, and that a lowering of basal plasma insulin levels occurred concomitantly with the hyperglycaemia. Two subsequent studies have reproduced these findings [3,8]. The threshold level for the effect of galanin in the dog was thereby found to be between 0.25 and 2.5 pmol/kg per min [3]. To study whether the lowering of basal plasma insulin levels induced by galanin is due to a direct effect on the pancreas, the peptide was infused directly into the pancreatic artery at a dose level of 0.25 pmol/kg per min. Under such conditions, pancreatic insulin output decreased markedly (by 71 ± 2%), indicating that the observed lowering of basal plasma insulin levels in dogs is actually mediated by a direct inhibition of pancreatic insulin secretion [3].

The effect of intravenously administered galanin on basal plasma levels of glucose and insulin has also been investigated in man, rats and mice. In man, however, galanin did not affect basal plasma glucose or insulin levels when infused at either 7.8 or 33.2 pmol/kg per min [9]. In rats an intravenous infusion of galanin between 4 and 32 pmol/kg per min lowered basal plasma insulin levels but was without any effect on basal plasma glucose levels [10]. Furthermore, at 5 min after an intravenous injection of galanin (2 nmol/kg) there was a marked reduction in basal portal insulin levels [11], and at 3 min after an intravenous injection of the peptide at 22 nmol/kg, peripheral basal insulin levels were lowered [12]. In mice, galanin, injected intravenously at dose levels between 0.53 and 8.5 nmol/kg, induced hyperglycaemia and hypoinsulinaemia [13]. Thus, studies performed in vivo in dogs and mice show that galanin induces hyperglycaemia and hypoinsulinaemia, whereas in rats hypoinsulinaemia without hyperglycaemia is seen, and in man, no influence has been observed on either basal plasma insulin or glucose levels.

3. STIMULATED PLASMA INSULIN LEVELS

A few studies have been directed to the question of whether galanin, besides the lowering of basal plasma insulin levels, also inhibits stimulated plasma insulin levels. In dogs, intravenous infusion of galanin (20 pmol/kg per min) has thereby been shown to inhibit the plasma insulin response to intravenous administration of glucose or arginine or the oral administration of glucose or a mixed meal [7,14]. In rats, intravenous infusion of galanin (4–32 pmol/kg per min) has been demonstrated to reduce the plasma insulin responses to intravenously administered glucose or arginine [10], and intravenous injections of galanin (2–22 nmol/kg) have been shown to reduce glucose-stimulated insulin secretion but to be without influence on the insulin response to gastric inhibitory polypeptide (GIP) [12]. Furthermore, in mice, intravenous injection of galanin (1.1 or 4.3 nmol/kg) inhibits the plasma insulin responses to intravenously administered glucose, carbachol (cholinergic agonist), terbutaline (β_2-adrenoreceptor agonist), or the octapeptide of cholecystokinin (CCK-8) [13,15]. In man, however, galanin infusion (33.2 pmol/kg per min) did not affect the glucose-induced increase in plasma insulin levels, although the glucose clearance was reduced by the peptide [9].

4. INSULIN SECRETION IN VITRO

Since the infusion of galanin in the pancreatic artery markedly reduced basal pancreatic insulin output in dogs [3], it may be concluded that galanin lowers basal plasma insulin levels and reduces the insulin responses under a variety of stimulatory conditions by inhibiting insulin secretion. This conclusion has been supported by several in vitro studies. Thus, in the perfused canine pancreas, galanin (1 pM–10 nM) markedly reduced insulin secretion at 5.5 or 11.1 mM glucose in either the presence or absence of arginine (5 mM) [16], and in the perfused rat pancreas, galanin (5 μM) reduced insulin secretion during administration of 5.5 mM glucose [17]. Also, in the latter case similar effects were obtained irrespective whether arginine (10 mM) was present. Furthermore, galanin has also been demonstrated to inhibit insulin secretion from cultured rat islets (at

28 nM galanin and 6.7 mM glucose) [11] and from freshly isolated rat islets (galanin at concentrations of 10 pM–1 μM and glucose at concentrations of 5.5–16.7 mM) [10]. Theoretically, the inhibitory effects of galanin on insulin release from both the perfused pancreas and isolated islets might be indirect. However, a direct inhibition by galanin of insulin secretion was finally demonstrated in a study on single pancreatic mouse β-cells (at 16 nM galanin and 20 mM glucose) [6]. Thus, studies presented so far clearly demonstrate that galanin is a potent inhibitor of both basal and stimulated insulin secretion in dogs, rats and mice, both in vivo and in vitro. Such an effect is likely, at least in part, to be exerted by a direct effect on the islet β-cells.

5. SOMATOSTATIN SECRETION

Pancreatic somatostatin secretion in dogs in vivo has been demonstrated to be inhibited by galanin [3]. Also somatostatin secretion from the perfused canine pancreas in vitro has been shown to be markedly suppressed by galanin [16]. In dogs, the sensitivity to galanin of the somatostatin cells has been found to be slightly lower than that of the β-cells, since the threshold dose for galanin to inhibit insulin secretion was between 1 and 10 pM, whereas the corresponding value for somatostatin secretion was between 10 and 100 pM [16]. In contradistinction to these findings in the dog, a single study on the perfused rat pancreas showed no influence of galanin (5 μM) on somatostatin secretion [17].

6. GLUCAGON SECRETION

Initially, no influence on plasma glucagon levels was observed during intravenous infusion of galanin in dogs [7]. This finding was later confirmed in another study in dogs [8] and in work in man [9] as well as in rats [10]. However, by the use of the more sensitive technique for determination of glucagon secretion in vivo, measurement of changes in plasma glucagon levels in the pancreatic vein during intrapancreatic administration of galanin, a stimulation of glucagon secretion could be demonstrated in dogs [3]. Similarly, intravenously administered galanin, at high dose levels, increased plasma glucagon levels in mice [13]. However, recently performed studies in vitro have not been able to confirm this conclusion, since galanin did not affect glucagon secretion from the perfused rat pancreas [17] and at high dose levels (10 nM), galanin was even found to inhibit glucagon secretion from the perfused canine pancreas [16]. To what extent these apparent discrepancies can be accounted for by species differences is at the moment not known. However, differences due to experimental conditions should not be overlooked. In fact, in dogs galanin stimulates glucagon secretion in vivo [3] but inhibits it in vitro [16]. A possible explanation to this discrepancy might be a variation in sensitivity of the somatostatin cells to galanin. Noteworthy is that somatostatin secretion seems to be inhibited by galanin at comparatively lower doses in vivo [3] than in vitro [16], and consequently, that glucagon secretion might be more readily stimulated in vivo due to lower local levels of somatostatin.

7. PANCREATIC POLYPEPTIDE (PP) SECRETION

During intravenous galanin infusion in man (33.2 pmol/kg per min), plasma levels of PP were reduced [9] indicating an inhibition of PP secretion. Since galanin in the same experiment did not affect plasma insulin levels [9] it could be concluded that in man, the secretion of PP is more sensitive to galanin than is that of insulin. A recent study on the perfused rat pancreas has, similarly, shown that galanin inhibits PP secretion [18].

8. MECHANISM OF ACTION

From what has been discussed so far, it is obvious that the most pronounced effect of galanin in the endocrine pancreas is its inhibition of insulin release. Since galanin inhibits insulin release evoked by various secretagogues, such as glucose, arginine, carbachol, terbutaline, cholecystokin as well as oral intake of glucose or mixed meal [7,10,12,13], it is likely that galanin stimulation interferes with a central step in the insulin secretory machinery. When trying to establish the exact mechanisms, it has been demonstrated that neither does the peptide influence pancreatic venous levels of noradrenaline [3] nor are its effects on insulin release affected by adrenergic blockade [16].

Hence, it is not likely that the effects of galanin on the β-cells are mediated through either activation of adrenergic nerves or interaction with endogenous adrenoceptors. That the effects of galanin indeed can be accounted for, at least in part, by a direct interaction with the pancreatic β-cells has recently been demonstrated, using a suspension of β-cells isolated from obese, hyperglycaemic, mice [6]. Since insulin release is a Ca^{2+} regulated process, it is of interest to note that galanin-induced inhibition of glucose-stimulated insulin release is paralleled by both a repolarization and a reduction in free cytoplasmic Ca^{2+} concentration, $[Ca^{2+}]_i$ [6]. The reduction in $[Ca^{2+}]_i$ is probably not due to a direct interference with the voltage-activated Ca^{2+} channels, since there is no effect of galanin when these channels are opened by depolarization induced by high concentrations of K^+ [6]. Hence, the reduction in $[Ca^{2+}]_i$ probably results from a repolarization-induced closure of the voltage-activated Ca^{2+} channels. Noteworthy is that galanin has similar effects to the hyperglycaemic sulphonamide diazoxide on membrane potential, $[Ca^{2+}]_i$ and insulin release [6,19]. The pronounced repolarization induced by both diazoxide and galanin is difficult to reconcile with anything but an activation of some sort of K^+ channels. With regard to diazoxide, it is indeed clearly established that this compound exerts its repolarizing effect through an activation of the ATP-regulated K^+ channels in β-cells [19,20]. To what extent the repolarizing effects of galanin can be explained in terms of a similar activation of the ATP-regulated K^+ channels has up to now been more uncertain. However, preliminary data [21] indicate that galanin actually activates K^+ channels of similar characteristics to those regulated by ATP [19], i.e. a single conductance of about 60 pS. Whether galanin in addition activates some other types of K^+ channels can so far just be a matter of speculation. The reduction in $[Ca^{2+}]_i$ subsequent to the repolarization is then probably, at least to a major extent, responsible for the suppression of insulin release. However, at this point we cannot exclude the possibility that galanin also interacts with more distal steps in the stimulus-secretion coupling.

In fig.1 the possible mechanisms whereby galanin evokes its inhibitory effect on insulin release are outlined. The decrease in $[Ca^{2+}]_i$ might

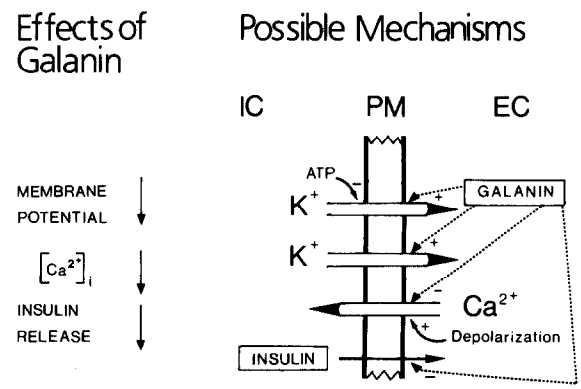

Fig.1. Effects of galanin on pancreatic β-cells and the possible mechanisms whereby these effects are exerted. IC, PM, and EC denote intracellular, plasma membrane, and extracellular, respectively. Solid arrows represent known mechanisms whereas dashed arrows indicate possible mechanisms.

result from either a repolarization-induced closure of the voltage-activated Ca^{2+} channels, implying that galanin activates some sort of K^+ channels, or a direct interference with the Ca^{2+} channels, although, as discussed above, the possibility that galanin inhibits the insulin secretory mechanism at a step distal to the regulation of $[Ca^{2+}]_i$ should not be overlooked. Hence, the final understanding of both the exact mechanisms whereby galanin regulates membrane potential and $[Ca^{2+}]_i$ and the extent to which a reduction in $[Ca^{2+}]_i$ can explain the interference with the insulin secretory mechanism awaits future more sophisticated biochemical, cell biological and electrophysiologial studies.

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