Stimulative effect of substance P on insulin secretion from isolated rat islets under normobaric oxygen incubation

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ABSTRACT The effects of substance P (SP), physalaemin, and [D-Pro², D-Phe⁷, D-Trp⁹]-SP on insulin release from isolated, cultured rat islets were investigated. Substance P stimulated insulin secretion in a dose-dependent manner at 0.1-100 nmol/L under one atmosphere of air with glucose 2.75, 5.5 and 20 mmol/L in the culture medium. Physalaemin 100 nmol/L was added to the culture medium, also stimulated insulin secretion. [D-Pro², D-Phe⁷, D-Trp⁹]-SP 10 nmol/L reversed the stimulative effect of substance P. However, substance P 1 nmol/L inhibited insulin secretion from isolated rat islets under hyper-baric oxygen condations.

KEY WORDS substance P; physalaemin; [D-Pro², D-Phe⁷, D-Trp⁹] substance P; islands of Langerhans; insulin; cultured cells

Substance P is an undecapeptide found in high concentrations in the brain and intestine. The peptide causes a wide variety of effects both *in vitro* and *in vivo*, including excitation of neurons[1], particularly nociceptive neurons; stimulation of gut contraction and salivation[2]; and antagonism to morphine analgesia[3]. Substance P is not only in the intrinsic nerve plexus, but also in enterochromaffin cells, especially in the duodenum of the gastrointestinal tract[4,5].

Although the effects of substance P on the endocrine pancreas have been studied in various laboratories using mouse[6], rat [7-10], and dog[11-14], consistent results have not been obtained. In our preliminary experiments, we found that substance P could stimulate insulin secretion from isolated rat islets under one atmosphere of air. However, a previously study reported that substance P inhibited insulin secretion from rat islets under hyper-baric oxygen[9]. The present study was designed to observe the effect of substance P on insulin secretion from isolated rat islets under normo- and hyper-baric oxygen conditions. The substance P agonist physalaemin and an antagonist, [D-Pro², D-Phe⁷, D-Trp⁹]-SP were also used in an attempt to elucidate the stereospecific effects of substance P on rat islets.

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Materials and methods

Islet isolation and culture Male wistar rats (Charles River, Canada) weighing 275 ± SD 20 g were anesthetized by ip sodium pentobarbital (55 mg/kg, M. T. C. Pharmaceuticals, Ontario). Islets were isolated from the pancreas of the rats by a collagenase (Type V, Sigma) digestion technique[15] and hand picked with the aid of a dissecting microscope. The isolated islets (200/flask) were then cultured for 3 h or over night in 10 ml of CMRL-1969 medium (Connanught Laboratories Ltd., Willowdale, Ontario) supplemented with glucose 5.5 mmol/L, 7.5% (vol/vol) fetal bovine serum, and 50 μg/ml gentamicin in a humidified atmosphere containing 5% CO_2 at 37℃. In the first series of experiments, after pre-incubation the islets were rinsed 3 times with CMRL-1969 medium and removed into 12×75 mm culture tubes. Islets (10 islets per tube) were incubated for 1 h at 37℃ in a Dubnoff shaker (60 strokes/min) under one atmosphere of air. Added to each

tube : 1 ml of CMRL-1969, graded concentrations of glucose (2.75, 5.5, and 20 mmol/L), substance P (Sigma: 0.1, 1, 10, 100 nmol/L), physalaemin (Sigma: 100 nmol/L) or [D-Pro^2, D-Phe^7, D-Trp^9]-SP (Sigma: 10 nmol/L), and bovine serum albumin 1 mg/ml (Sigma). For further verifying the effect of substance P, 10 islets were incubated in 1 ml Krebs-Ringer bicarbonate buffer with aprotinin 1000 kIU (KRB, containing glucose 5.5 mmol/L bubbled with 95% O_2 + 5% CO_2 mixture gas, pH 7.4), with or without substance P 1 nmol/L. Incubated samples were then kept frozen at −20℃ one week for radioimmunoassay of insulin concentration.

In the second series of experiments, 10 islets per tube were preincubated in 1 ml KRB buffer for 1 h at 37℃ under 95% O_2 + 5% CO_2. After 1 h of preincubation the medium was withdrawn from the tube and replaced by fresh KRB buffer containing aprotinin 1000 kIU, with or without substance P 1 nmol/L, and then islets were incubated under 95% O_2 + 5% CO_2 for another hour. After the second hour of incubation, the media were then taken for insulin determination.

Hormone assays The insulin content of the culture medium was determined by radioimmunoassay with solid-phase ^{125}I-insulin, using antibody-coated tubes (Diagnostic Products Corp, USA).

Statistical analysis The statistical significance of the results was determined by a Student's two-tailed *t* test. All values are expressed as the mean±SD of the number of observations indicated.

Results

In culture medium with a glucose concentration of 5.5 mmol/L, substance P increased insulin secretion from islolated rat islets in a dose-dependent fashion (Fig 1). With the addition of substance P 0.1 and 1 nmol/L to the medium, insulin secretion was only slightly increased. However, in medium containing 10 and 100 nmol/L of substance P, a significantly higher amount of insulin was secreted ($P<0.05$).

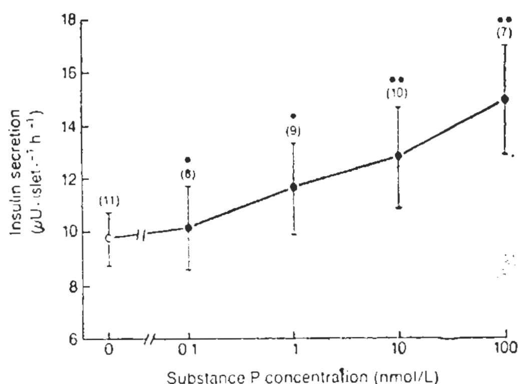

Fig 1. Stimulative effect of various doses of substance P on insulin secretion from rat islets after 1 h incubation in CMRL-1969 medium. The number within parentheses indicates the number of tubes (10 islets/tube) per group. (○) Control group, glucose (5.5 mmol/L) in CMRL-1969 alone. (●) Test group, glucose (5.5 mmol/L) in CMRL-1969 adding various concentrations of substance P. $^*P>0.05$, $^{**}P<0.05$ *vs* glucose alone.

The stimulative effects of substance P 100 nmol/L on insulin secretion in response to graded concentrations of glucose in the culture medium were also observed (Fig 2). The insulin release was increased as the glucose level was raised from 2.75 to 20 mmol/L. Moreover, the insulin release was still increased considerably by substance P in concentrations of glucose from 2.75 to 20 mmol/L in culture medium.

Fig 3 illustrates a comparison of the effects of substance P, [D-Pro^2, D-Phe^7, D-Trp^9]-SP and physalaemin on insulin release from the isolated rat islets. Physalaemin 100 nmol/L, as a homologue of substance P, also effectively stimulated significant release of insulin in the medium containing glucose 5.5 mmol/L ($P<0.01$), whereas, interestingly, this stimulative effect of substance P could be reversed completely by the presence of substance P antagonist

[D-Pro^2, D-Phe^7, D-Trp^9]-SP 10 nmol/L ($P<0.01$). It is noteworthy that [D-Pro^2, D-Phe^7, D-Trp^9]-SP alone did not produce any significant changes in insulin secretion in KRB buffer containing glucose at 5.5 mmol/L

Fig 4 illustrates a comparison of the effects of substance P under different conditions of incubation. On the left side of the solidline, islets were incubated in KRB buffer containing glucose 5.5 mmol/L and 1000 kIU aprotinin for 1 h at 37℃ in a Dubnoff shaker (60 strokes/min) under one atmosphere of air. Substance P significantly stimulated insulin secretion from isolated islets as compared with control ($P<0.05$). On the right side of the solidline, islets were incubated in KRB buffer containing glucose 5.5 mmol/L and aprotinin 1000 kIU for 1 h at 37℃ under 95% O_2 + 5% CO_2. With the addition of substance P 1 nmol/L to the medium, insulin secretion decreased only slightly (on the left side of dotted of line). In another parallel experiment, islets were incubated in the same condition as cited above, substance P was added during the

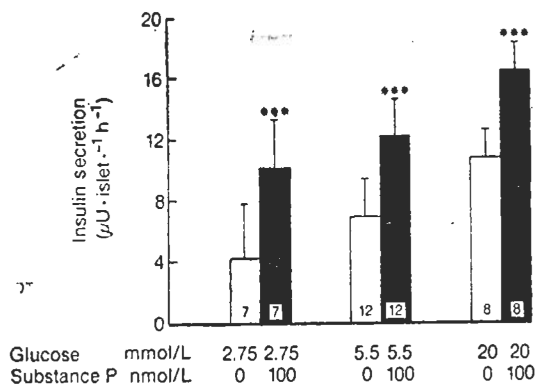

Fig 2. Stimulative effect of substance P on glucose-induced insulin secretion from isolated islets. In this and subsequent figures, the number within the bars represents the number of tubes (10 islets/tube) per group. ***$P<0.01$ *vs* glucose alone.

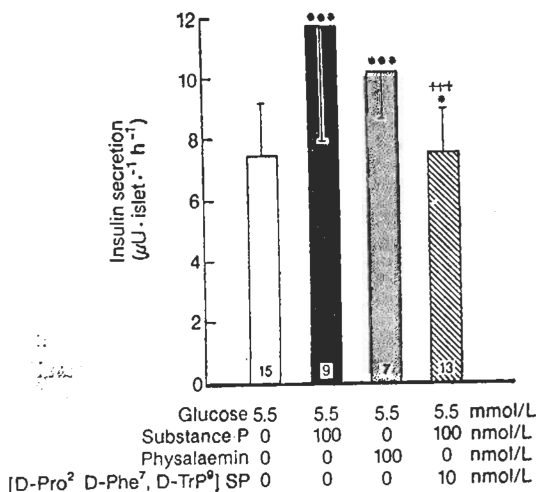

Fig 3. The effects of substance P, physalaemin and [D-Pro^2 D-Phe^7, D-Trp^9]-SP on insulin secretion from rat islets. *$P>0.05$, ***$P<0.01$ *vs* glucose 5.5 mmol/L in CMRL-1969 alone. †††$P<0.01$ [D-Pro^2, D-Phe^7, D-Trp^9]-SP (10 nmol/L) plus substance P (100 nmol/L) *vs* substance P (100 nmol/L)

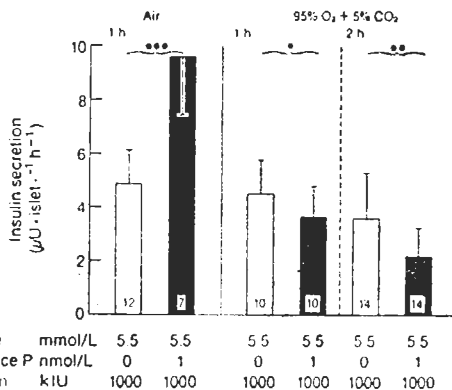

Fig 4. The opposite effects of substance P on insulin secretion from rat islets under one atmosphere of air and 95% O_2 + 5% CO_2. On the left side of the solidline: islets were incubated in 1 ml KRB buffer containing glucose 5.5 mmol/L with or without substance P 1 nmol/L at 37℃ in a Dubnoff shaker (60 strokes/min) under one atmosphere of air. On the right side of the solid-line: islets were incubated in 1 ml KRB buffer containing glucose 5.5 mmol/L with or without substance P 1 nmol/L at 37℃ under 95% O_2 + 5% CO_2 for 1 h. In other experiments, islets were incubated under the same condition as cited above for 2 h, substance P was added during the second hour of incubation (on the right side of the dotted line) *$P>0.05$, **$P<0.05$, ***$P<0.01$.

second hour of incubation, the suppressive effect of substance P on insulin secretion was significant ($P<0.05$, on the right side of dotted line).

Discussion

This study clearly demonstrates that substance P has a stimulative effect on glucose-induced insulin secretion from isolated rat islets under normobaric oxygen condition of incubation, both in CMRL-1969 medium and KRB buffer. The study also shows that the agonist of substance P, physalaemin has a stimulative effect on insulin release identical to that of substance P, whereas the antagonist of substance P, [D-Pro^2, D-Phe^7, D-Trp^9]-SP, specifically antagonizes the effect of substance P on insulin release from rat islets. In addition, substance P could depress insulin secretion from isolated rat islets under high baric oxygen incubation. The latter result comfirms the previous work by Moltz *et al*[9]. The reason for opposite effects of substance P on insulin secretion produced by incubation with different conditions is not clear. However, it seems to us that the responsiveness to substance P of the rat islets might be greatly influenced by the environmental oxygen partial pressure. As for the *in vivo* effect of substance P, it was reported that substance P inhibited insulin release from the rat pancreas[8,10]. From their data, we found that the secretion of insulin was only initially depressed for less than 10 min, and then tended to return to control level, or the insulin curve even had a tendency to be increased[8]. Regarding the present study, we determined insulin secretion from isolated rat islets only after incubation for 1 h. It is quite possible that the inhibitory effect of substance P on insulin secretion at the initial short time course was not observed under the conditions of our experiment.

However, in the dog experiment, Kaneto *et al*[11] infused substance P at a dose of 20 pmol/(kg • min) for 10 min into the cranial pancreaticoduodenal artery of an anesthetized dog and found that during the infusion, blood flow and plasma concentrations of both glucagon and insulin in the cranial pancreaticoduodenal vein increased promptly. Hermansen[14] observed that, at concentrations of 0.2-0.5 nmol/L, substance P stimulated the secretion of somatostatin, insulin and glucagon from the isolated perfused pancreaticoduodenal canine preparation. In other words, substance P seems to have a rather complex effect on glucose-induced insulin secretion. Our results are consistent with the view that substance P, at the concentrations which may be delivered to the pancreatic islets by the circulation, could directly stimulate the secretion of insulin[10]. This could have some physiological siginfiicance. However, the mechanism of the effects of substance P on insulin release remains to be determined.

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常氧条件下P物质刺激离体大鼠胰岛的胰岛素分泌

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提要　离体胰岛在含有葡萄糖，P物质，菲萨拉明(physalaemin)和[D-脯², D-苯丙⁷, D-色⁹]P物质的培养液中孵育1 h(37℃，空气)．用放射免疫法测定培养液中的胰岛素含量．P物质0.1-100 nmol/L可刺激离体大鼠胰岛的胰岛素释放并呈剂量-效应关系．菲萨拉明同样刺激胰岛素的释放．[D-脯², D-苯丙⁷, D-色⁹] P物质可反转P物质刺激胰岛素分泌增加的作用．然而，在高氧条件下P物质抑制离体大鼠胰岛的胰岛素释放．

关键词　P物质；菲萨拉明；[D-脯², D-苯丙⁷, D-色⁹]P物质；胰岛；胰岛素；细胞培养

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