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Pertussis toxin reverses the inhibition of insulin secretion caused by [Arg^8]vasopressin in rat pancreatic islets

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When rat pancreatic islets were incubated with 10^{-8} M arginine vasopressin in the presence of 15 mM glucose there was a pronounced inhibition of insulin release in comparison with controls. This inhibitory effect appeared to be specific for vasopressin since it was antagonised by vasopressin antibody. Moreover, pertussis toxin (100 ng/ml) reversed the inhibition of insulin release due to vasopressin, indicating the possible involvement of a guanyl-nucleotide regulatory protein in the inhibitory effect. Nevertheless, 10^{-8} M vasopressin increased islet concentrations of cyclic AMP even under conditions where insulin release was decreased.

Vasopressin; cyclic AMP; Pertussis toxin; Islet of Langerhans

1. INTRODUCTION

Vasopressin exhibits a variety of metabolic effects in addition to its well known antidiuretic and vasopressor activities. Among cells affected are hepatocytes, adrenal glomerulosa cells and human platelets. Tissue vasopressin receptors have also been extensively studied, and it has been suggested that there is more than one type of receptor involved in the mode of action of this hormone. There is also much evidence to implicate a guanyl-nucleotide regulatory protein in the action of vasopressin [1,2].

There has, however, been uncertainty concerning the effect of vasopressin on the islets of Langerhans. There are contradictory reports as to how vasopressin affects insulin release either in vivo [3–5], or in vitro [6].

We have re-investigated how vasopressin affects islet function in vitro. Our present study on the islets reveals that vasopressin in physiological concentrations persistently inhibits insulin secretion in the presence of stimulatory concentration of glucose. We have further investigated how vasopressin may affect cyclic AMP levels in islets and whether the inhibitory response to vasopressin is reversed by treatment with pertussis toxin.

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2. MATERIALS AND METHODS

Islets of Langerhans were isolated from male Sprague-Dawley rats [7] (body wt 150–200 g). Ten islets were incubated with either 5 or 15 mM glucose with or without [Arg^8]vasopressin (Sigma) at a concentration of 10^{-8} M in oxygenated bicarbonate buffer containing 0.1% bovine serum albumin. After incubation for 1 h at 37°C in a shaking waterbath, insulin was measured in the incubation medium by radioimmunoassay (RIA) [8].

[Arg^8]Vasopressin antibody (Biogenesis), at a dilution 1:10 of the commercial preparation, was used with vasopressin at a concentration of 0.5×10^{-11} M. The hormone and its antibody were incubated together for 1 h then added to the islets and incubated for a further hour. After the incubation period, insulin secretion was measured by RIA. Pertussis toxin (kindly donated by Dr Irons of Porton International) was incubated with the islets overnight at a concentration of 100 ng/ml in tissue culture

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medium RPMI 1640 and 5 mM glucose. The islets were then washed with fresh bicarbonate buffer and incubated with [Arg^8]vasopressin for 1 h. Cyclic AMP was measured by immunoassay [9] using a kit obtained from New England Nuclear. In the assay 15 islets were incubated in each tube for 10 min with 10^{-8} M [Arg^8]vasopressin.

3. RESULTS

In the present study vasopressin was incubated with the islets of Langerhans at 2 different concentrations of glucose (table 1). The insulin response to 15 mM glucose was inhibited by vasopressin but this was not so at a basal glucose concentration (5 mM). The inhibitory action of vasopressin on insulin secretion seems to be specific for the vasopressin molecule. This is also shown in table 1, where the results of incubating [Arg^8]vasopressin with islets in the presence of vasopressin antibody are shown. The inhibitory effects are abolished. Furthermore, when the islets were incubated overnight with 100 ng/ml pertussis toxin (table 2) and then incubated with vasopressin, the inhibitory effect of vasopressin was reversed even in the presence of high concentrations of glucose. When pertussis toxin is incubated with islets alone, there is a small stimulation of insulin release above the control values. Although vasopressin inhibited insulin release, it increased cyclic AMP concentration in islets (table 3), at both 5 and 15 mM glucose.

Table 1

Effect of [Arg^8]vasopressin on islet insulin release with and without vasopressin antibody

Additions to incubation medium	Insulin release (pg/islet per min)
Expt 1	
glucose (5 mM)	1.80 ± 0.19 (10) a
glucose (5 mM) + vasopressin (10^{-8} M)	1.70 ± 0.13 (6)
glucose (15 mM)	6.32 ± 0.06 (11) b
glucose (15 mM) + vasopressin (10^{-8} M)	3.65 ± 0.25 (10) c
Expt 2	
glucose (5 mM)	0.91 ± 0.09 (5) d
glucose (15 mM)	2.70 ± 0.22 (5) e
glucose (15 mM) + vasopressin (10^{-11} M)	1.59 ± 0.10 (5) f
glucose (15 mM) + vasopressin (10^{-11} M) + vasopressin antibody	2.67 ± 0.16 (5) g

For differences between a and b, b and c, d and e, e and f and f, and g, $p < 0.001$. Isolated rat islets were incubated as shown. The amount of insulin secreted during the incubation period was determined by RIA. Values given are means ± SE. Number of observations are shown in parentheses

Table 2

Effect of pertussis toxin treatment on [Arg^8]vasopressin inhibition of insulin secretion

Additions to incubation medium	Insulin secretion (pg/islet per min)	
	Control	Toxin treated (100 ng/ml)
5 mM glucose	2.92 ± 0.47 (5)	3.56 ± 0.43 (5)
5 mM glucose + 10^{-8} M vasopressin	2.06 ± 0.35 (5)	4.14 ± 0.74 (5)
15 mM glucose	21.98 ± 0.97 (5)	25.08 ± 0.68 (5) a
15 mM glucose + 10^{-8} M vasopressin	12.64 ± 0.85 (5)	23.05 ± 1.26 (5) b

For differences between control and toxin-treated islets (a) $p < 0.02$, (b) $p < 0.001$. For other differences between these two groups, $p > 0.05$. Isolated rat pancreatic islets were preincubated with pertussis toxin overnight (100 ng/ml) then washed and incubated as in text for 1 h. Insulin secreted during incubation period was determined by RIA. Values are means ± SE. Number of observations are shown in parentheses

Table 3

Cyclic AMP levels in [Arg^8]vasopressin-treated rat islets

Additions to incubation medium	Cyclic AMP (fmol/islet per min)
5 mM glucose (control)	10.72 ± 0.65 (5)
5 mM glucose and 10^{-8} M vasopressin	22.50 ± 1.10 (5) a
15 mM glucose (control)	15.82 ± 1.80 (5)
15 mM glucose and 10^{-8} M vasopressin	21.98 ± 0.81 (5) b

For differences between appropriate controls and values a and b, $p < 0.02$. Groups of 15 islets/tube were incubated for 10 min as shown, then taken for RIA to measure cyclic AMP levels. Values are means ± SE. Number of observations are shown in parentheses

4. DISCUSSION

The inhibitory effect of [Arg^8]vasopressin on glucose-stimulated insulin release was unexpected. In tissues such as liver, vasopressin produces a rise in cytosolic calcium. If vasopressin had behaved this way in islets, then the rise in calcium would be expected to increase insulin release.

However, other studies in vivo have also suggested that vasopressin may inhibit insulin release. Thus, in dogs, it has been shown that vasopressin inhibits the rise in plasma insulin caused by oxytocin [4]. Earlier experiments on the effects of vasopressin on islets in vitro were inconclusive [6].

Moreover, the effect of vasopressin is seen at physiological concentrations (0.5×10^{-11} M). The inhibitory action of vasopressin on insulin release at this concentration was reversed by adding a specific [Arg^8]vasopressin antibody to the medium. In addition, a similar inhibition of glucose-stimulated release was shown in the presence of [Lys^8]vasopressin (not shown). This adds weight to the idea that the inhibitory effect is a feature of the vasopressin molecule.

However, the mechanism of inhibition is uncertain. Since it is reversed by pertussis toxin, vasopressin may be exercising its inhibitory effect through a guanyl-nucleotide regulatory protein. Several other reports indicate that vasopressin appears to exert its effects by this means [10,11].

Despite its inhibitory effect on insulin release, [Arg^8]vasopressin was able to increase cyclic AMP accumulation in islets. There have been conflicting reports about the effect of vasopressin on cyclic AMP accumulation in various tissues. Thus, in liver, vasopressin alone had no effect on cyclic AMP levels [12,13]. By contrast, it has recently been shown that vasopressin was able to increase cyclic AMP in smooth muscle cell culture [14]. Islets of Langerhans appear to resemble smooth muscle in this respect.

It is generally supposed that there are two types of receptors by which vasopressin is functional in tissues. These have been designated V_1 and V_2 type receptors [15]. The V_2 receptor operates through adenylate cyclase and the V_1 receptor is assumed to act through the phosphatidylinositol system involving changes in cytosolic Ca^{2+} concentrations [16–18]. Our results suggest that the V_2 type of receptor may be operative in islets. Presumably to exercise its inhibitory effect, vasopressin is not associated with the classical V_1 receptor, already described in other tissues.

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