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Atrial natriuretic factor has a weak insulinotropic action in the isolated perfused rat pancreas

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Summary. The effect of atrial natriuretic factor (ANF; 1, 10, 100, 1000 pmol/l) on insulin release from the isolated perfused rat pancreas was studied. ANF weakly augmented the glucose (10 mmol/l)-stimulated insulin release during the second (controls: 100%; 1 pmol/l: 99%; 10 pmol/l: 149%, $P < 0.05$; 100 pmol/l: 111%; 1000 pmol/l 135%), but not the first phase of the secretory response. In contrast, the first, but not the second phase of arginine (10 mmol/l)-stimulated insulin release was significantly enhanced by ANF (1000 pmol/l; controls: 100%; 1000 pmol/l: 235%, $P < 0.05$). The hormone did not influence basal insulin secretion. Our data indicate an insulinotropic effect of ANF on the rat pancreas, which is dependent on the utilized background secretagogues.

Key words: ANF – Insulin secretion – Perfused rat pancreas – Glucose – Arginine – Basal insulin release

Introduction

Atrial natriuretic factor (ANF), originally isolated from heart atria, has been identified in many organs, including the gastrointestinal tract [2, 3, 8, 12, 17, 18]. The hormone exerts a wide spectrum of metabolic and cardiovascular effects. Furthermore, receptors for ANF have been identified in a wide variety of tissues [1], including blood vessels [9, 13], cardiac tissue [10], lung [11], exocrine pancreas [2], and rat adipocytes [6]. Recently, specific receptors for ANF were demonstrated on RINm5F cells, an insulinoma-derived cell line [15], and on isolated rat islets [16]. This was of particular interest, because it has been found that exogenous ANF increases circulating insulin levels in man [14]. However, ANF had no influence on basal and stimulated insulin release from RINm5F cells

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(stimulated by glyceraldehyde [15]) or isolated rat islets (stimulated with glucose [16]).

In the present study, the effect of ANF on basal (unstimulated) and on glucose, and arginine-induced insulin secretion was re-investigated utilizing the isolated perfused rat pancreas. This was of interest, in as much as by using this model the integrity of the whole gland is guaranteed. Furthermore, the isolated perfused rat pancreas is more sensitive to secretagogues than isolated islets.

Materials and methods

Substances

Synthetic rat atrial natriuretic factor (28 amino acids) was from Peninsula (St. Helens, Merseyside, UK), bovine serum albumin (fraction V) from Serva (Heidelberg, FRG), and aprotinin (Trasylol) from Bayer (Leverkusen, FRG). All other chemicals used were analytical grade and were obtained from Merck (Darmstadt, FRG).

Animals

Male albino Wistar rats (180–240 g), kept in a light- und temperature-controlled room, were fed a standard diet (Altromin, Lage, FRG) and had free access to water.

Perfusion experiments

Rats were each anesthetized by an i.p. injection of sodium pentobarbitone (45 mg/kg body weight). The pancreas, spleen, stomach, and proximal part of the duodenum were perfused through cannulated abdominal aorta and coeliac axis as described before [7] and detailed previously [4]. The entire preparation was removed from the cadaver and placed into a perfusion chamber (37°C). The perfusion medium consisted of a Krebs-Ringer-bicarbonate buffer supplemented with 0.2% bovine serum albumin (pH 7.4 when gassed with 95% O_2 and 5% CO_2). Venous effluent was collected on aprotinin (1000 U/fraction) in intervals by a cannula inserted into the portal vein. The perfusion pump generated a constant flow rate of 5 ml/min. After a 10-min period for equilibration at 2.8 mmol/l glucose, the medium was changed to buffer supplemented with glucose, arginine, and ANF in concentrations as indicated.

Analytical determinations

Insulin in perfusate was measured by radioimmunoassay [5]. The standard was a mixture of rat insulin I and II and was obtained from Novo (Mainz, FRG).

Statistics

Insulin secretion is expressed as nanograms of insulin per minute (Figs. 1, 2) and was further calculated as integrated hormone release during the first (0–8 min) and the second (9–44 min) phase of the insulin secretory response (Tables 1, 2). Data are given as means ± SEM of (n) experiments. For statistical analysis the U-test of Wilcoxon, Mann, and Whitney was used. Statistical significance was set at the 5% level.

Results

A typical biphasic insulin-secretion pattern was observed when the pancreata were perfused with medium containing 10 mmol/l glucose (Fig. 1). When ANF

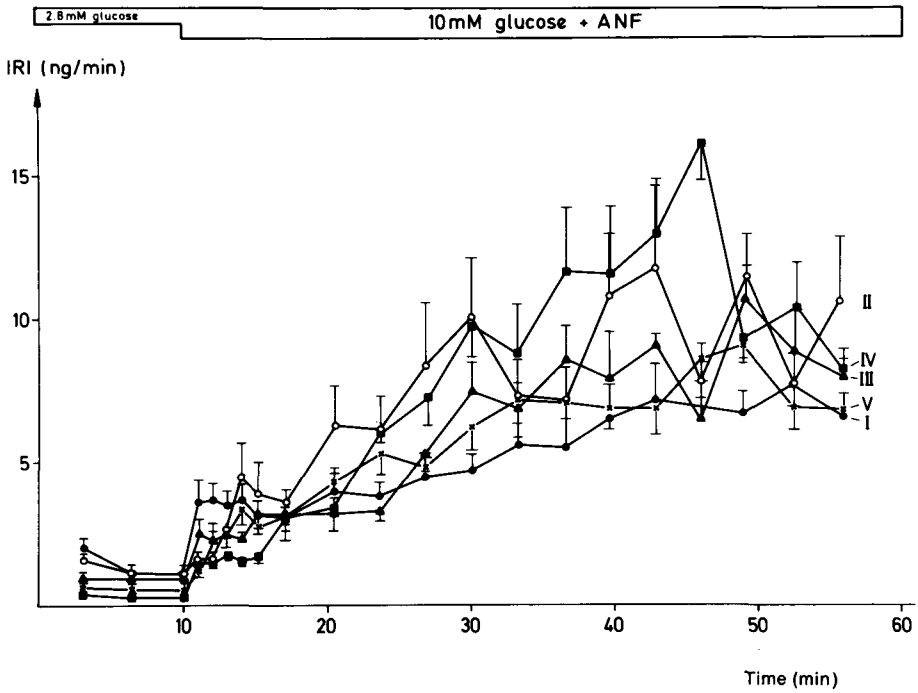

Fig. 1. Effect of 1, 10, 100, and 1000 pmol/l ANF on glucose- (10 mmol/l) induced insulin secretion from the isolated perfused rat pancreas. Data are given as means ± SEM of (*n*) experiments. The first phase of insulin release corresponds to 10–18 min (0–8 min for the stimulation period) and the second phase to 19–54 min (9–44 min for the stimulation period): *I*, 10 mmol/l glucose (controls); $n = 20$ (●–●); II, 10 mmol/l glucose + 1000 pmol/l ANF; $n = 6$ (○–○); III, 10 mmol/l glucose + 100 pmol/l ANF; $n = 5$ (▲–▲); IV, 10 mmol/l glucose + 10 pmol/l ANF; $n = 4$ (■–■); V, 10 mmol/l glucose + 1 pmol/l ANF; $n = 5$ (×–×)

Table 1. Effect of ANF (1, 10, 100, and 1000 pmol/l) on integrated glucose (10 mmol/l)-stimulated insulin secretion during the first (nanograms of insulin/0–8 min) and the second phase (nanograms of insulin/9–44 min) from the isolated perfused rat pancreas. Data given as means ± SEM of (*n*) experiments

Glucose (mmol/l)	ANF (pmol/l)	*n*	First phase	Second phase
2.8	–	4	8.2 ± 1.0	33.1 ± 2.5
10	–	20	29.1 ± 3.0[a] (100%)	234.3 ± 41.4[a] (100%)
10	1	5	24.2 ± 5.0 (83%)	232.8 ± 16.0 (99%)
10	10	4	17.1 ± 1.6 (59%)	348.3 ± 40.9[b] (149%)
10	100	5	22.6 ± 3.6 (78%)	261.2 ± 21.3 (111%)
10	1000	6	27.1 ± 6.8 (93%)	317.3 ± 76.6 (135%)

[a] Significantly different from experiments with 2.8 mmol/l glucose
[b] Significantly different from experiments with 10 mmol/l glucose

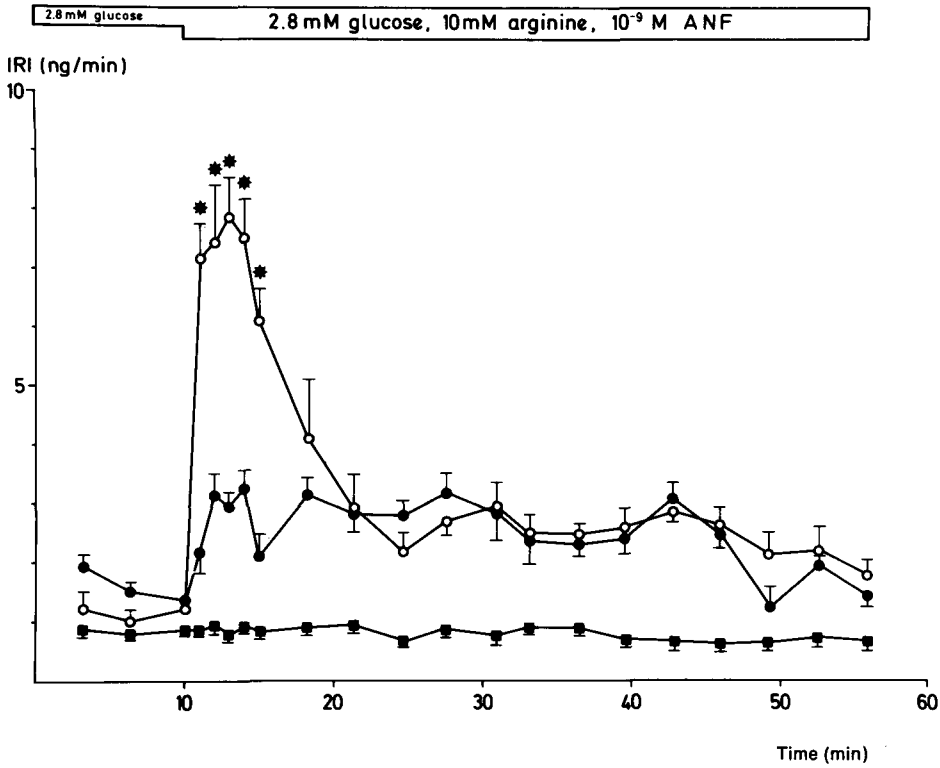

Fig. 2. Effect of ANF (1000 pmol/l) on arginine- (10 mmol/l, plus 2.8 mmol/l glucose)-induced insulin secretion from the isolated perfused rat pancreas. Data are given as means ± SEM of (*n*) experiments. The first phase of insulin secretion corresponds to 10–18 min (0–8 min during the stimulation period) and the second phase to 19–54 min (9–44 min during the stimulation period): 2.8 mmol/l glucose; $n = 4$ (■–■); 2.8 mmol/l glucose + 10 mmol/l arginine; $n = 8$ (●–●); 2.8 mmol/l glucose + 10 mmol/l arginine + 1000 pmol/l ANF; $n = 6$ (○–○)

Table 2. Effect of ANF (1000 pmol/l) on basal (2.8 mmol/l glucose) and (10 mmol/l) arginine-stimulated integrated insulin secretion during the first (nanograms of insulin/0–8 min) and the second phase (nanograms of insulin/9–44 min) from the isolated perfused rat pancreas. Data are given as mean ± SEM of *(n)* experiments

Glucose (mmol/l)	Arginine (mmol/l)	ANF (pmol/l)	*n*	First phase	Second phase
2.8	–	–	4	8.2 ± 1.0 (100%)	33.1 ± 2.5 (100%)
2.8	–	1000	6	7.8 ± 0.7 (95%)	31.2 ± 1.1 (106%)
2.8	10	–	6	21.2 ± 4.9 (100%)[a]	81.6 ± 4.8 (100%)[a]
2.8	10	1000	6	49.9 ± 7.4 (235%)[a, b]	64.4 ± 2.7 (79%)[a]

[a] Significantly different from experiments with 2.8 mmol/l glucose
[b] Significantly different from experiments with 2.8 mmol/l glucose + 10 mmol/l arginine

was introduced, this biphasic pattern of insulin release remained unaltered. ANF at a concentration of 10 pmol/l significantly increased the glucose (10 mmol/l)-stimulated insulin release during the second [controls: 234.3 ± 41.4 ng insulin/9–44 min (100%); 10 pmol/l ANF: 348.3 ± 40.9 ng insulin/9–44 min (149%); $P < 0.05$] but not during the first secretion period (controls: 29.1 ± 3.0 ng insulin/0–8 min (100%); 10 pmol/l ANF: 17.1 ± 1.6 ng insulin/0–8 min (59%). Higher ANF levels (100, 1000 pmol/l) and ANF at 1 pmol/l had no significant effect on glucose (10 mmol/l)-induced insulin secretion (Fig. 1, Table 1).

At 1000 pmol/l, ANF enhanced the arginine-induced insulin secretion during the first [controls: 21.2 ± 4.9 ng insulin/0–8 min (100%); 1000 pmol/l ANF: 49.9 ± 7.4 ng insulin/0–8 min (235%); $P < 0.05$], but not during the second phase of the insulin secretory response (Fig. 2; Table 2). ANF had no effect on basal (unstimulated) insulin release (Table 2).

Discussion

Although ANF and ANF receptors have been demonstrated in several organs, which suggests a wide spectrum of actions, in most of these tissues the biological effects are virtually unknown [1–3, 10, 16–18]. Recently, specific receptors for ANF were demonstrated on rat insulinoma-derived cells and on isolated rat islets [15, 16]. In both systems, ANF induced an increase of intracellular cGMP after binding to its receptor, but did not affect insulin release [15, 16]. These results contrast to those of studies in man, where infusion of ANF elevates plasma insulin levels [14].

Data recorded in the present study now demonstrate a weak insulinotropic action of ANF at the isolated perfused rat pancreas. We observed an enhanced insulin secretory response of glucose (10 mmol/l)- and arginine (10 mmol/l)-stimulated insulin release. However, clear dose-dependency could not be established. Nevertheless, at 10 pmol/l ANF, the integrated insulin secretory response to 10 mmol/l glucose calculated for the second phase of the insulin release was significantly above that in controls (Table 1). Furthermore, when the dynamic responses of the whole perfusion experiment were considered, significant differences from controls (10 mmol/l glucose) for single points were revealed in all groups.

Our data are at variance with earlier results in comparable investigations [15, 16]. This can be explained by the use of different systems: it is well established that isolated islets after collagenase digestion are less sensitive to secretagogues than the isolated perfused pancreas, where the integrity of the gland is maintained. Furthermore, indirect effects of ANF not localized at the B-cell could also contribute to a weak insulinotropic action. Our results coincide with studies in man, where infusion of ANF elevated plasma insulin levels [14].

ANF influenced the two periods of the typical biphasic insulin secretory response to glucose or arginine in different ways: in the presence of glucose it augmented the second, but not the first period, and with arginine only the first phase was modulated (Figs. 1, 2). Activation of different metabolic pathways within the B-cell could be involved in these actions.

From a critical point of view, all the observations obtained up to now do not allow a definitive conclusion as to whether ANF plays a physiological role in reg-

ulating insulin release. Therefore, the data now presented should stimulate further investigations of a possible significance of ANF for the glucose homeostasis.

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