

Amino acid transformation of oscillatory Ca^{2+} signals in mouse pancreatic β -cells

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Abstract

Glucose-induced increase of cytoplasmic Ca^{2+} in pancreatic β -cells is usually manifested as slow oscillations from the basal level. The significance of this rhythmicity for maintaining normal β -cell function with periodic variations of circulating insulin made it of interest to investigate how the oscillatory Ca^{2+} signal was affected by various amino acids. Individual mouse β -cells were very sensitive to alanine, glycine and arginine, sometimes responding with a transformation of the oscillations into sustained elevation of cytoplasmic Ca^{2+} at amino acid concentrations as low as 0.1 mM. Stimulation of the entry of Ca^{2+} , obtained either by raising the extracellular

concentration or by prolonging the open state of the voltage-dependent Ca^{2+} channels with BAY K 8644, resulted in reappearance of the rhythmic activity in the presence of the amino acids. Oscillatory Ca^{2+} signals in intact islets were more resistant to transformation by amino acids than those of individual β -cells. It is therefore suggested that signals from the adjacent cells make it possible for β -cells situated in islets to overcome a suppression of the oscillatory activity otherwise seen in the presence of alanine, glycine or arginine.

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Introduction

The concentration of insulin in the blood varies in a periodic fashion (Jaspan *et al.* 1986, Lefèbvre *et al.* 1987), reflecting the pulsatile release of the hormone from the pancreas (Goodner *et al.* 1991, Pørksen *et al.* 1995). A step towards understanding the mechanisms of this release was the observation that individual pancreatic mouse β -cells respond to glucose stimulation with low frequency (0.2–0.5/min) oscillations of cytoplasmic Ca^{2+} (Grapengiesser *et al.* 1988). Subsequent studies indicated that these slow oscillations can be accounted for by periodic depolarization of the β -cells with accompanying entry of Ca^{2+} (Hellman *et al.* 1992). The glucose-induced pulses of insulin release from rodent (Gilon *et al.* 1993, Bergsten *et al.* 1994) as well as human (Hellman *et al.* 1994a) islets are known to result from a synchronization of the oscillatory Ca^{2+} signals in the β -cells.

Analyses of individual β -cells revealed that the glucose-induced rise of the cytoplasmic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) is sometimes manifested as a sustained elevation (Grapengiesser *et al.* 1988, Hellman *et al.* 1992, 1994a). Moreover, minor damage of the β -cells results in a disappearance of the slow oscillations with preservation of a glucose response in terms of a sustained rise of $[\text{Ca}^{2+}]_i$ (Grapengiesser *et al.* 1990). The observation that the oscillatory state is sensitive to various disturbances is consistent with reports that the regular variations of

circulating insulin disappear during the development of Type 1 (Bingley *et al.* 1992) and Type 2 (O'Rahilly *et al.* 1988) diabetes.

Studies of individual β -cells have indicated that the amino acids glycine, sarcosine and non-metabolizable α -aminoisobutyric acid, which are co-transported with Na^+ , can transform the glucose-induced oscillations of $[\text{Ca}^{2+}]_i$ into a sustained elevation (Tengholm *et al.* 1992, McClenaghan *et al.* 1997). It will now be shown that alanine and arginine have similar effects as glycine in transforming the oscillations and that the rhythmicity can be restored by promoting Ca^{2+} entry. Individual β -cells were more sensitive to the suppression of the oscillatory Ca^{2+} signal than those situated in islets, sometimes responding to amino acid concentrations as low as 0.1 mM.

Materials and Methods

Materials

Reagents of analytical grade and deionized water were used. Collagenase, Hepes and bovine serum albumin were purchased from Boehringer Mannheim (Mannheim, Germany) and fetal calf serum was supplied by Gibco (Paisley, Strathclyde, UK). Poly-L-lysine came from Sigma Chemical Co (St Louis, MO, USA). Fura-2

acetoxymethyl ester and Pluronic F-127 were obtained from Molecular Probes Inc (Eugene, OR, USA) and (\pm) BAY K 8644 was provided by Calbiochem (La Jolla, CA, USA).

Preparation of pancreatic islets and β -cells

Islets of Langerhans were isolated from adult obese-hyperglycemic mice from a local non-inbred colony (Hellman 1965), using collagenase digestion. Previous studies have established that these islets consist of more than 90% β -cells, which respond adequately to glucose and other regulators of insulin release (Hahn *et al.* 1974). The islets were either kept in culture for 1–4 days in RPMI 1640 medium supplemented with 10% fetal calf serum or dissociated into single cells by shaking in a Ca^{2+} -deficient medium (Lernmark 1974). The single cells were allowed to attach to circular 25 mm cover glasses during 1–4 days culture in the RPMI 1640 medium. Unless stated otherwise subsequent experimental handling of islets and cells was performed with a basal medium buffered with 25 mM HEPES and containing 3 mM glucose, 0.5 mg/ml bovine serum albumin, 125 mM NaCl, 5.9 mM KCl, 1.2 mM $MgCl_2$ and 1.3 mM $CaCl_2$. NaOH was used for adjusting the pH to 7.4.

Measurements of cytoplasmic Ca^{2+} in individual β -cells

The cells were loaded with fura-2 during 30–40 min incubation at 37 °C with 0.5 μ M of its acetoxymethyl ester. The cover glasses with the attached cells were then used as bottoms of an open chamber containing 160 μ l medium. The chamber was placed on the stage of an inverted microscope within a climate box maintained at 37 °C and the cells were superfused at a rate of 0.75 ml/min. The microscope was equipped with an epifluorescence illuminator and a 100 X UV fluorite objective. A filter changer of a time-sharing multichannel spectrophotofluorometer (Chance *et al.* 1975) provided excitation light flashes at 340 and 380 nm and the emission was measured at 510 nm with a photomultiplier. Calibration was performed as previously described (Gryniewicz *et al.* 1985), assuming a K_d for the Ca^{2+} fura-2 complex of 224 nM. Selection of β -cells for analyses was based on previously described criteria (Berts *et al.* 1995).

Measurements of cytoplasmic Ca^{2+} in intact islets

Loading of islets with fura-2 was accomplished by 45 min incubation at 37 °C with 2 μ M of its acetoxymethyl ester together with 0.02% (w/v) Pluronic F-127. The islets were then allowed to attach to cover glasses coated with poly-L-lysine, making it possible to perform similar types of superfusion experiments as used for the single β -cells (see above). The analyses were restricted to islets responding to 11 mM glucose with slow oscillations of $[Ca^{2+}]_i$.

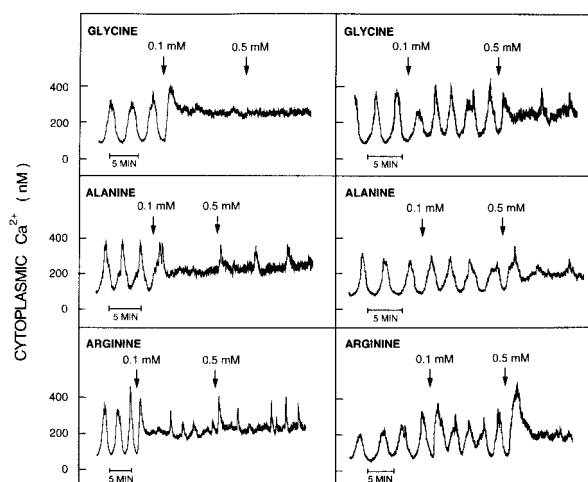


Figure 1 Effects of low concentrations (0.1 or 0.5 mM) of glycine, alanine and arginine on glucose-induced oscillations of cytoplasmic Ca^{2+} in individual β -cells. The arrows indicate the time point of the addition of the amino acids to a medium containing 11 mM glucose. Exposure to 0.1 mM amino acid resulted in transformation of the oscillations into sustained elevation in three out of twelve (glycine), three out of nine (alanine) and three out of nine (arginine) experiments. After raising the amino acid concentration from 0.1 to 0.5 mM the oscillations were transformed into sustained elevation in seven out of twelve (glycine), six out of nine (alanine) and five out of nine (arginine) experiments.

Measurements were made in an optical plane close to the lower surface of the islets. $[Ca^{2+}]_i$ was calculated without compensating for the autofluorescence, which was <15%. The addition of the amino acids did not affect the fluorescence excitation ratio in the absence of the fura-2 indicator.

Results

Individual β -cells with oscillations of $[Ca^{2+}]_i$ in response to 11 mM glucose were exposed to different concentrations of glycine, alanine or arginine. Each of these amino acids could transform the oscillations into sustained elevation of $[Ca^{2+}]_i$ when added at concentrations as low as 0.1 or 0.5 mM (Fig. 1). These responses were readily reversible (not shown). The amino acid effect was critically dependent on the extracellular Ca^{2+} concentration. A rise of Ca^{2+} from 1.3 to 10 mM restored the $[Ca^{2+}]_i$ rhythmicity even in the presence of much higher concentrations of the amino acids (Fig. 2). The oscillations obtained after raising extracellular Ca^{2+} were more prominent than the original ones and started from a level below the sustained elevation. The Ca^{2+} channel agonist BAY K 8644 (1 μ M) was also effective in promoting a reappearance of the $[Ca^{2+}]_i$ oscillations in β -cells exposed to glycine, alanine or arginine (Fig. 3).

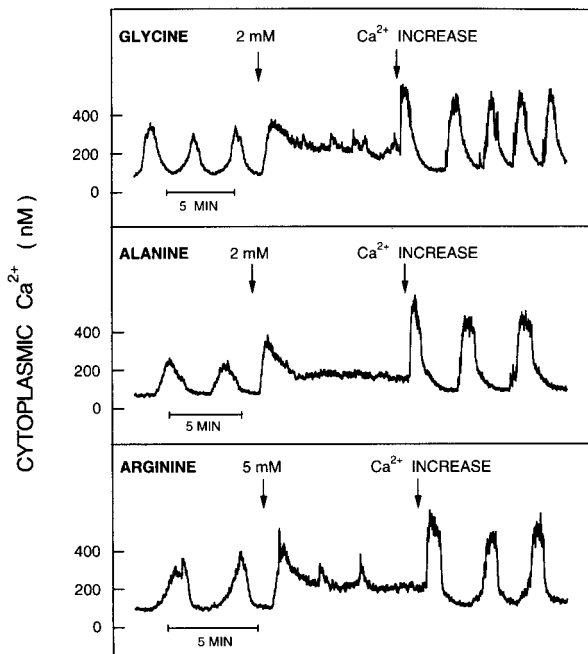


Figure 2 Transformation of glucose-induced oscillations of cytoplasmic Ca^{2+} in individual β -cells by glycine, alanine and arginine and reappearance of the rhythmic activity after subsequent rise of extracellular Ca^{2+} . The arrows indicate the time point of the addition of the amino acids and raising Ca^{2+} from 1.3 to 10 mM in a medium containing 11 mM glucose. The increase of the Ca^{2+} concentration resulted in reappearance of the oscillations in five out of eight (2 mM glycine), all of seven (2 mM alanine) and all of five (5 mM arginine) experiments.

The intact pancreatic islets reacted to the amino acids with transformation of the $[\text{Ca}^{2+}]_i$ oscillations into sustained elevation (Fig. 4). However, much higher concentrations were required compared with individual β -cells and even at 10 mM glycine, alanine or arginine only about 55% of the islets responded in this manner (Table 1).

Discussion

Previous studies of individual β -cells indicate that amino acids are important regulators of the slow $[\text{Ca}^{2+}]_i$ oscillations. Whereas addition of leucine had a similar effect as an increase of the glucose concentration in initiating the rhythmic activity (Grapengiesser *et al.* 1989), the $[\text{Ca}^{2+}]_i$ oscillations were transformed into sustained elevation by glycine and sarcosine (Tengholm *et al.* 1992) as well as by the non-metabolizable α -aminoisobutyric acid (McClenaghan *et al.* 1997). The latter three amino acids all accumulate in β -cells by co-transport with Na^+ (Hellman *et al.* 1971, Sehlin 1972). We now report that alanine, another amino acid transported with the Na^+ -dependent systems ASC and A (Christensen 1990), also converts the $[\text{Ca}^{2+}]_i$ oscillations into sustained elevation. These obser-

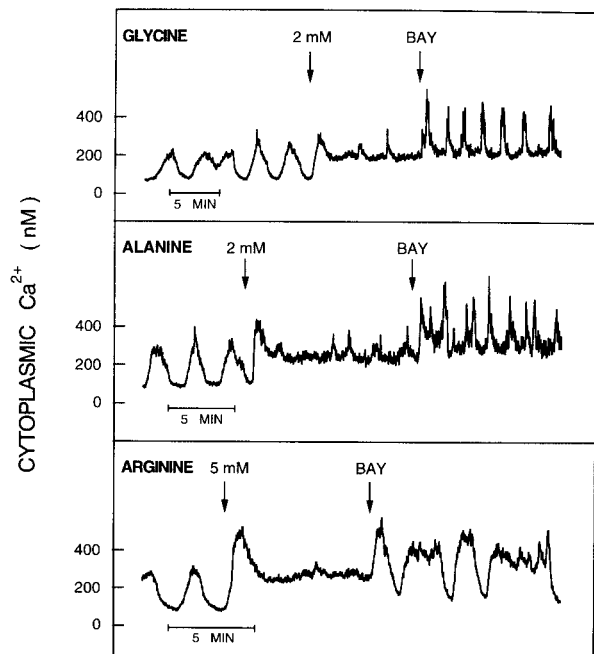


Figure 3 Transformation of glucose-induced oscillations of cytoplasmic Ca^{2+} in individual β -cells by glycine, alanine and arginine and reappearance of the rhythmic activity after subsequent addition of 1 μM BAY K 8644 to a medium containing 11 mM glucose. The exposure to BAY K 8644 resulted in reappearance of the oscillations in six out of seven (2 mM glycine), all of five (2 mM alanine) and all of five (5 mM arginine) experiments.

vations support the idea that the Na^+ permeability is an important factor whether a glucose-induced rise of $[\text{Ca}^{2+}]_i$ is manifested as oscillations or sustained elevation.

It is evident from electrophysiologic studies that alanine has a depolarizing action on insulin-releasing cells of tumor origin (Dunne *et al.* 1990) as well as on normal β -cells (Henquin & Meissner 1981). Moreover, it has been reported that a methylated derivative of α -aminoisobutyric acid elicits rapid depolarization with a prolonged rise of $[\text{Ca}^{2+}]_i$ in insulinoma cells (Dunne *et al.* 1990). Although α -aminoisobutyric acid promotes the influx of Na^+ , it has negligible effects on the sodium content of the β -cells due to the presence of an efficient Na^+/K^+ pump (McClenaghan *et al.* 1997). It is therefore likely that it is the depolarizing effect of the Na^+ entry rather than the intracellular accumulation of the ion which transforms oscillatory $[\text{Ca}^{2+}]_i$ into sustained elevation. The present data support this idea in demonstrating that such a transformation is not restricted to the amino acids co-transported with Na^+ but is also seen with the positively charged arginine. The latter amino acid is known to accumulate in mouse islet cells as efficiently as alanine (Hellman *et al.* 1971) with the aid of Na^+ -independent transporters of cationic amino acids (Smith *et al.* 1997).

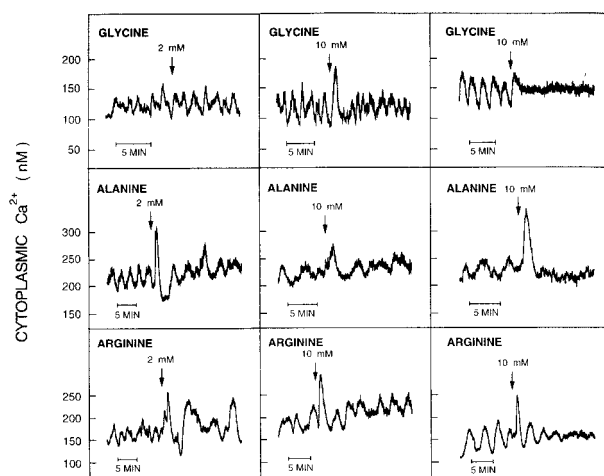


Figure 4 Effects of glycine, alanine and arginine on glucose-induced oscillations of cytoplasmic Ca²⁺ in intact islets. The arrows indicate the time point of the addition of the amino acids to a medium containing 11 mM glucose. The oscillatory activity often persisted in the presence of 2 mM (panels to the left) or 10 mM (middle panels) of the amino acids. Examples of transformation of the oscillatory activity into sustained elevation are shown in the panels to the right. The proportion of islets responding for each of the amino acids is presented in Table 1.

There is convincing evidence that this electrogenic transport accounts for the depolarization resulting in the increase of [Ca²⁺]_i required for the arginine potentiation of glucose-stimulated insulin release (Henquin & Meissner 1981, Blachier *et al.* 1989). In the light of recent comparisons with other cationic amino acids (Smith *et al.* 1997) it seems unlikely that the generation of nitric oxide contributes to the effect of arginine on the secretory activity.

We now show that β-cells are very sensitive to glycine, alanine and arginine, sometimes responding with a disappearance of the oscillatory activity already at 0.1 mM. This concentration is lower than that found for each of the amino acids in mouse blood plasma, where the total amino acid content has been estimated to 2.5–2.6 mM (Martin & Soria 1995). Amino acid transformation of [Ca²⁺]_i

Table 1 Amino acid transformation of glucose-induced oscillations of cytoplasmic Ca²⁺ into sustained elevation in intact islets. [Ca²⁺]_i oscillations were induced by raising the glucose concentration from 3 to 11 mM. The proportion of islets responding to 2 or 10 mM amino acids with transformation of the oscillatory activity is indicated

Amino acid tested	Proportion of responding islets	
	(2 mM)	(10 mM)
Glycine	2/9	5/9
Alanine	0/6	6/11
Arginine	1/7	6/11

oscillations similar to that obtained in the present type of β-cells is seen with β-cells isolated from NMRI mice (M Ahmed, unpublished observations). Insulin, like other hormones, is released in pulses generated by oscillatory cytoplasmic Ca²⁺ (Hellman *et al.* 1992). It may therefore appear surprising that concentrations of amino acids well below those existing in the blood can suppress the oscillations of [Ca²⁺]_i. By extending the studies to whole islets it became possible to get further insight into this problem. Apparently, the rhythmicity is more stable in β-cells situated in islets, as indicated from our observation that suppression of the slow islet oscillations of [Ca²⁺]_i requires higher concentrations of the amino acids. The present data do not allow definite conclusions on why the glucose-induced [Ca²⁺]_i oscillations are more resistant to amino acids in intact islets than in isolated β-cells. The [Ca²⁺]_i oscillations in a syncytium of islet cells are determined not only by the metabolism of the individual β-cells but also by synchronizing signals from adjacent cells (Hellman *et al.* 1994b). It is likely that these signals make it possible for the β-cells to overcome the suppression of the oscillatory activity otherwise obtained in the presence of low concentrations of glycine, alanine or arginine.

Previous studies have shown that the glucose-induced slow oscillations of [Ca²⁺]_i are critically dependent on the rate of Ca²⁺ entry (Eberhardson *et al.* 1996). A lowering of extracellular Ca²⁺ below 0.8 mM resulted in the disappearance of the oscillatory activity. Moreover, it was possible to induce oscillations in β-cells responding to glucose with a sustained elevation of [Ca²⁺]_i, by raising extracellular Ca²⁺ or prolonging the open state of the voltage-dependent Ca²⁺ channels with BAY K 8644. We now provide additional arguments for the importance of influx rate in the generation of slow [Ca²⁺]_i oscillations, demonstrating that both a rise of extracellular Ca²⁺ or addition of BAY K 8644 re-establishes a rhythmicity suppressed by amino acids. It is open for discussion how the promotion of the Ca²⁺ entry evokes transformation of the sustained elevation of [Ca²⁺]_i into oscillations. Increase of [Ca²⁺]_i above a certain level can be expected to have negative feed-back effects on further entry of the ion mediated by direct inhibition of the voltage-dependent Ca²⁺ channels and/or hyperpolarization resulting from an augmented K⁺ permeability. The latter effect may not only be due to activation of Ca²⁺-dependent K⁺ channels but also reflect the opening of ATP-regulated K⁺ channels due to lowering of the ATP/ADP ratio in the β-cell periphery following the consumption of energy required for the enhanced extrusion of Ca²⁺. With the observation that experimentally induced suppression of the rhythmicity in the Ca²⁺ signaling can be overcome by promotion of the Ca²⁺ entry it will be important to decide whether impaired influx of this ion contributes to the loss of the regular cycles of plasma insulin release known to occur during the development of Type 2 diabetes (O'Rahilly *et al.* 1988).

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