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# Glucose-induced oscillations of intracellular $Ca^{2+}$ concentration resembling bursting electrical activity in single mouse islets of Langerhans

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Intracellular $Ca^{2+}$ levels were monitored in single, acutely isolated mouse islets of Langerhans by dual emission Indo-1 fluorometry. High-frequency (3.1 $min^{-1}$) $[Ca^{2+}]_i$ oscillations with a brief rising time (1–2 s) and 10 s half-width ('fast' oscillations) were detected in 11 mM glucose. Raising the glucose concentration to 16.7 mM increased the duration of these oscillations, which were otherwise absent in 5.5 mM glucose. $[Ca^{2+}]_i$ waves of lower frequency (0.5 $min^{-1}$) and longer rising time ('slow' oscillations) were also recorded. The data indicate that "fast" oscillations are directly related to $\beta$-cell bursting electrical activity, and suggest the existence of extensive networks of electrically coupled cells in the islet.

Islet of Langerhans; Indo-1; $Ca^{2+}$ concentration, intracellular; $Ca^{2+}$ oscillation; Electrical activity; Intercellular coupling

## 1. INTRODUCTION

The importance of $Ca^{2+}$ as the primary second messenger in glucose-induced insulin secretion from the pancreatic $\beta$-cell is well established [1]. Although the exact mechanism whereby glucose activation of cell metabolism is coupled to $Ca^{2+}$ fluxes is not well understood, glucose stimulation is known to inhibit $\beta$-cell $K^+$ conductance [2], which in turn depolarizes the cells and activates voltage-sensitive $Ca^{2+}$ channels [3]. The ensuing $Ca^{2+}$ influx gives rise to an increase in intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) [4].

Glucose-induced electrical activity has long been known to occur in the form of rhythmic 'bursts' of $Ca^{2+}$ spikes at intermediate glucose concentrations (7–17 mM). If $Ca^{2+}$ regulatory mechanisms in the $\beta$-cell were efficient enough to handle rapidly the cytosolic $Ca^{2+}$ loads generated by these bursts of electrical activity, $[Ca^{2+}]_i$ would be expected to undergo oscillations of similar duration and frequency [5]. A $[Ca^{2+}]_i$ oscillatory activity has indeed been recorded from isolated mouse $\beta$-cells [6], but its characteristics – both in terms of frequency and time course – are not easily reconciled with those of the pattern of electrical activity measured in microdissected mouse islets of Langerhans. For example, the frequency of such oscillations is typically of 0.3 $min^{-1}$ [6], while the membrane potential oscillates at a pace of ~2–4 $min^{-1}$ (rarely below 1 $min^{-1}$) [7]. These basic discrepancies have led us to search for $[Ca^{2+}]_i$ oscillations in pancreatic $\beta$-cells arranged spacewise as identically as possible as cells in their native environment. We report in this study two types of $[Ca^{2+}]_i$ oscillations in acutely isolated mouse islets of Langerhans. One type – found in the majority of the experiments – has the expected characteristics of being produced by the islet bursting electrical activity. In addition, very low frequency oscillations were recorded in some experiments that resemble those found by other authors in isolated $\beta$-cells.

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## 2. MATERIALS AND METHODS

Mouse islets of Langerhans were isolated by collagenase digestion as previously reported [8]. Once isolated, islets were kept at 37°C in modified Krebs medium supplemented with 11 mM glucose and 3% bovine serum albumin.

Intracellular $Ca^{2+}$ was monitored by measuring the fluorescence emitted by the fluorophore Indo-1 [9]. Islets were loaded with Indo-1 by incubation for 30 min at room temperature with 2 $\mu$M of the acetoxymethyl derivative Indo-1/AM (Molecular Probes, OR, USA). This was added as a concentrated stock in a mixture of DMSO and 10% (w/w) Pluronic F 127 (Molecular Probes, OR, USA). The loaded islet was transferred to an experimental bath (vol. ~300 $\mu$l) mounted on the stage of a Nikon epifluorescence inverted microscope and superfused at a rate of 1 ml/min.

Indo-1 was excited at 350 ± 5 nm by means of a 100 W mercury lamp and a narrow band-pass filter. In order to minimize photobleaching of the indicator and photodamage to the preparation, neutral density filters were placed in the excitation pathway. Emitted light was split into two beams, which passed through band-pass filters (centered at 410 ± 5 nm and 480 ± 5 nm) and were finally detected by two photomultipliers (Thorn EMI 9924B). An increase in $[Ca^{2+}]_i$ produces a rise in Indo-1 fluorescence at 410 nm; conversely, a fluorescence decrease is observed at 480 nm (see fig.1) as expected from the spectral characteristics of this $Ca^{2+}$ indicator [9]. The ratio of Indo-1 fluorescence at both wavelengths was determined on-line, filtered at 10 Hz and stored on tape for further analysis. The advan-

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tages of using the fluorescence ratio rather than the individual signals for the measurement of $[Ca^{2+}]_i$ have been previously emphasized [9]. The fluorescence appeared to be homogeneously distributed throughout the islet.

We have attempted to calibrate the fluorescence signal in situ by using two calcium ionophores (ionomycin, Br-A23187) and found that neither adequately permeabilized the islets. This could conceivably be due either to the tight structure of the islets or to a genuine lack of ability of the ionophores to permeabilize the plasmalemma. When the fluorescence ratio was compared to an in vitro calibration curve, the values obtained for resting $Ca^{2+}$ concentration were around 80 nM (the peaks of oscillations recorded at 11 mM glucose were in the range 200–300 nM). Autofluorescence levels were measured from control islets and found to be less than 20% of the fluorescence emitted by Indo-1 in a typical experiment.

Experiments were carried out at 37°C with the islets bathed in a medium with the following composition (mM): 120 NaCl, 5 KCl, 25 $NaHCO_3$, 2.5 $CaCl_2$ and 1.1 $MgCl_2$. The medium was constantly gassed with a mixture of 95% $O_2$ and 5% $CO_2$ for a final pH of 7.4.

## 3. RESULTS

We have used the $Ca^{2+}$-chelating indicator Indo-1 to monitor $[Ca^{2+}]_i$ in single islets of Langerhans. This probe did not appear to affect islet function since the electrical activity recorded in the presence of 11 mM glucose remained unchanged after exposing islets to 1–5 $\mu$M Indo-1/AM for 30 min at 37°C (Santos, R.M., unpublished).

Fig.1 depicts typical changes in Indo-1 fluorescence recorded from an islet in the presence of 11 mM glucose. The upper and middle panels represent the fluorescence measured simultaneously at 410 and 480 nm, respectively. These signals change in opposite directions as expected from the spectral characteristics of Indo-1 (see section 2). The lower panel in fig.1 displays the ratio of fluorescence intensities at both wavelengths.

In the presence of glucose (11 mM), the fluorescence ratio (designated henceforth as $[Ca^{2+}]_i$ for simplicity) was found to oscillate at a frequency ranging from 2 to 5 $min^{-1}$ (fig.1, lower panel and fig.2, upper panel). A frequency histogram of these oscillations appeared to be described by a normal distribution (not shown) centered at 3.1 ± 0.8 $min^{-1}$ (± SD, $n$ = 17 experiments). The time-to-peak and half-width (time from half-rise to half-descent) of these oscillations were 1–2 s and 9.6 ± 5.5 s, respectively ($n$ = 18 experiments). $Ca^{2+}$ levels stayed elevated for a period of a few seconds following the rapid $[Ca^{2+}]_i$ rise of each individual oscillation; they subsequently decreased along an exponential-like time course (fig.1, lower panel). Short-lived transients were often seen during the plateau phase of these oscillations (see also fig.4). Since the cut-off frequency of the filter used in the recording amplifier was set at 10 Hz, the possible relationship between these $[Ca^{2+}]_i$ transients and individual calcium action potentials cannot be established.

The brief rising time ('fast') oscillations described above represented by far the most frequently observed

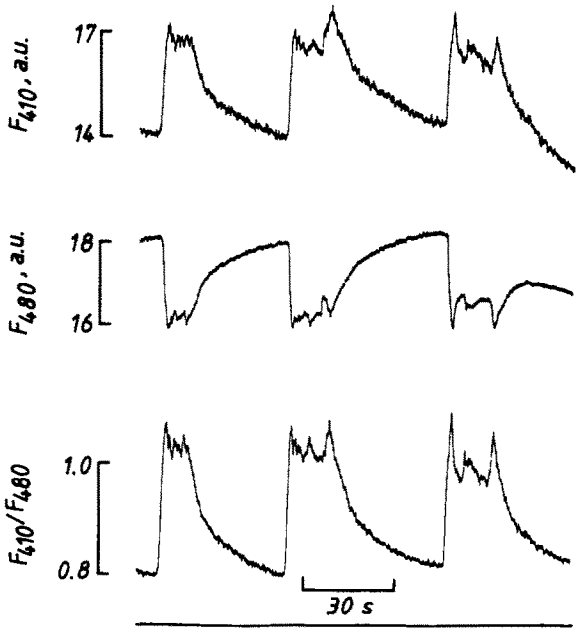


Fig.1. Oscillations of Indo-1 fluorescence recorded from a single islet of Langerhans in 11 mM glucose. Fluorescence (in arbitrary units, a.u.) at 410 nm (top panel) and 480 nm (middle panel) were simultaneously recorded and ratioed on-line (lower panel) as explained in section 2. An increase in $F_{410}/F_{480}$ denotes a rise in $[Ca^{2+}]_i$.

pattern (2/3 of the total). In addition, another type of oscillation was found which was clearly located to the left of the major population in the frequency histogram. Its frequency in 11 mM glucose averaged 0.5 ± 0.2 $min^{-1}$ ($n$ = 8; range 0.2–1 $min^{-1}$). These oscillations lasted typically for 1–3 min being the rising and the falling phase very smooth (fig.2, lower panel). Neither the duration nor the frequency of these 'slow' $[Ca^{2+}]_i$ oscillations were affected by glucose in the concentration range 11–22 mM (exposure times, 10–20 min). These oscillations disappeared once glucose concentration was reduced to sub-stimulatory levels (5.5 mM) (data not shown). A pure pattern of 'slow' oscillations was found in less than 1/3 of the islets probed. A mixed situation was also found occasionally as depicted in fig.2 (middle panel), where a 'slow' oscillatory pattern underlied 'fast' $[Ca^{2+}]_i$ oscillations. Interestingly, in this experiment the latter appeared on the positive slope of the background 'slow' waves.

We have investigated the glucose-dependence of the 'fast' $[Ca^{2+}]_i$ oscillations. Fig.3 illustrates the effect of increasing glucose concentration from a sub-stimulatory level (3 mM) to an intermediate level (11 mM). No $[Ca^{2+}]_i$ oscillations were recorded in 3 mM glucose, a condition in which the $\beta$-cell is also electrically silent [10]. Raising glucose concentration up to 11 mM resulted in a delayed but sharp increase in $[Ca^{2+}]_i$, which remained elevated for a period of 2–3 min. A rise in signal noise is apparent throughout this period, which is reminiscent of the initial phase of the biphasic

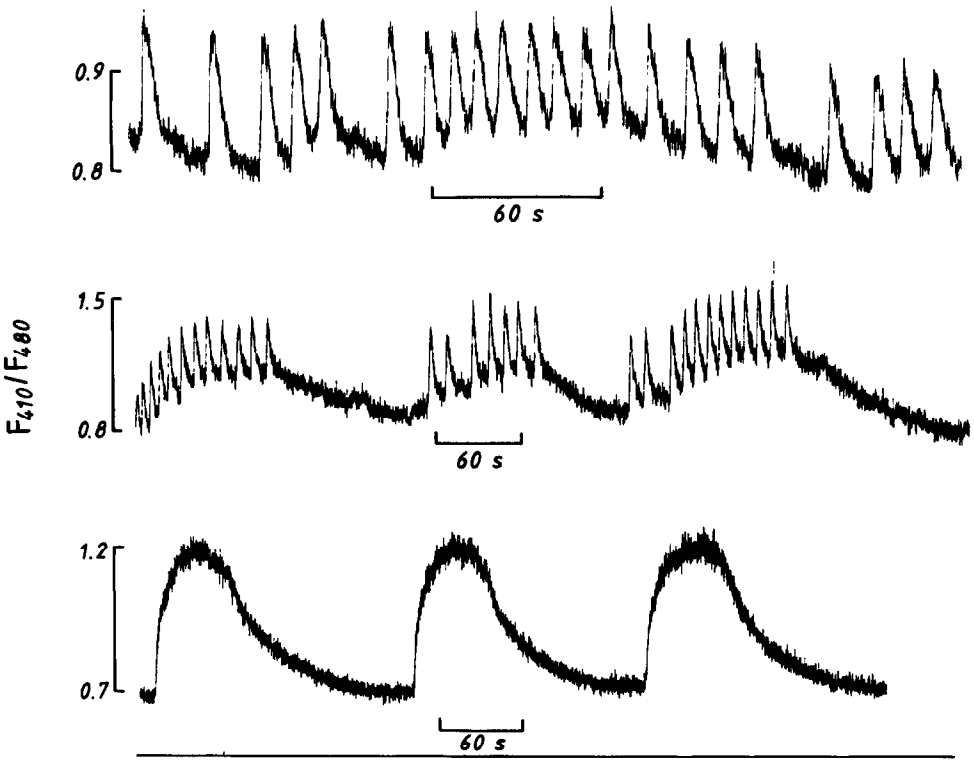


Fig.2. Two types of $[Ca^{2+}]_i$ oscillations recorded from 3 different islets in the presence of 11 mM glucose. The top panel shows a pattern of high-frequency oscillations with brief rising times ('fast' oscillations). The lower panel depicts a pure pattern of low-frequency, slowly developing ('slow') oscillations. The middle panel represents a mixed situation, with 'fast' oscillations superimposing on 'slow' $[Ca^{2+}]_i$ waves.

pattern of electrical activity observed when glucose concentration is increased from a low to a high level [11]. $[Ca^{2+}]_i$ then started to oscillate with a pattern similar to the one previously described in fig.1. Although the $Ca^{2+}$ level at the feet of these 'fast' oscillations initially decreased with time, it finally remained above the resting $Ca^{2+}$ level measured in 3 mM glucose. $[Ca^{2+}]_i$ decreased to pre-stimulatory levels following return to low glucose concentration. Another glucose concentration tested in the range 3–11 mM was 5.5 mM; because at this concentration the $[Ca^{2+}]_i$ did not differ from that at 3 mM (data not shown), we conclude that the glucose threshold for activation of $[Ca^{2+}]_i$ oscillations lies within the range 5.5–11 mM.

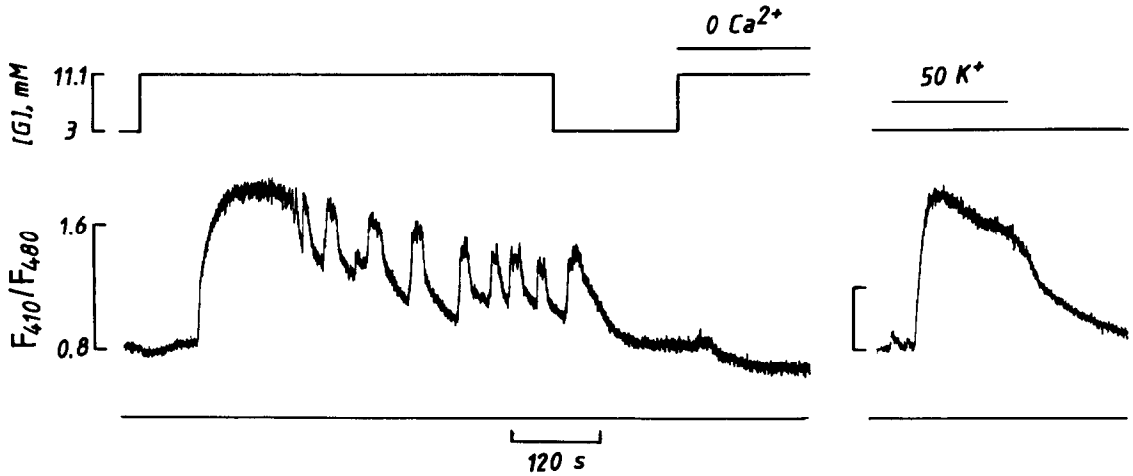


Fig.3. Effect of a rise in glucose concentration from 3 to 11 mM on $[Ca^{2+}]_i$ in the presence and in the absence of extracellular $Ca^{2+}$. Glucose concentration was changed in the presence of 2.5 mM $Ca^{2+}$ from 3 to 11 mM, and back to 3 mM, as depicted in the upper part of the figure (steps denote the times when solutions were switched with a stop-cock). Glucose concentration was also increased from 3 to 11 mM in the nominal absence of extracellular $Ca^{2+}$. The righthand part of the figure shows the effect of raising extracellular $K^+$ concentration to 50 mM at the expense of $Na^+$, in the same islet. The break in the record represents 16 min. Note the 2-fold vertical compression of the right record.

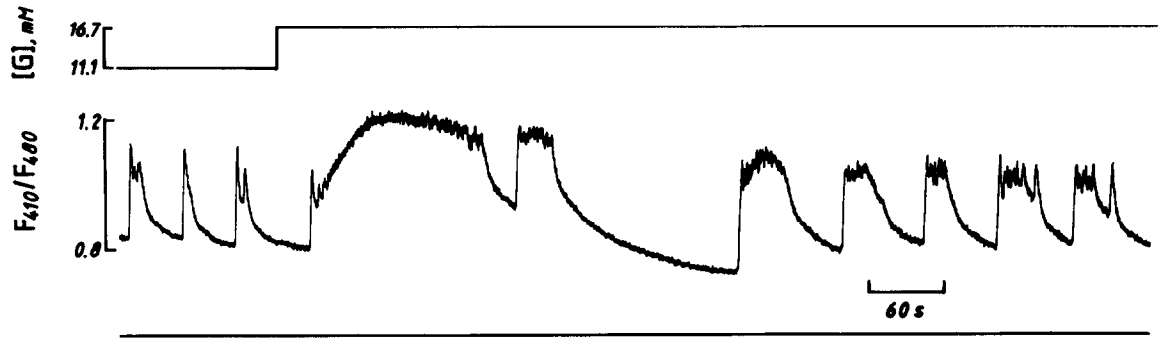


Fig.4. Effect of increasing glucose concentration from 11 to 16.7 mM on $[Ca^{2+}]_i$.

Since glucose-induced insulin release is dependent on extracellular $Ca^{2+}$ [12], we have examined the effect of glucose on $[Ca^{2+}]_i$ in the presence of $\mu$M extracellular $Ca^{2+}$ levels. Challenging the islet with 11 mM glucose in the nominal absence of $Ca^{2+}$ resulted in the blockade of the otherwise expected increase in $[Ca^{2+}]_i$ activity (fig.3). The $[Ca^{2+}]_i$ was actually slightly, but clearly decreased following exposure to 11 mM glucose. Identical results were obtained when extracellular $Ca^{2+}$ was removed 1 min before glucose concentration was raised, or in the presence of EGTA (0.5 mM) and no added $Ca^{2+}$ (data not shown). These data indicate that $Ca^{2+}$ influx is a necessary step for the appearance of the $[Ca^{2+}]_i$ oscillations. Since voltage-sensitive $Ca^{2+}$ channels are likely to be involved in this influx of $Ca^{2+}$, we attempted to detect $[Ca^{2+}]_i$ rises induced by depolarization. Raising extracellular $K^+$ concentration to 50 mM evoked a rapid increase in $[Ca^{2+}]_i$ as expected (fig.3). The spontaneous decrease in $[Ca^{2+}]_i$ observed in the continued presence of high $K^+$ could be due to either calcium-dependent inactivation of $Ca^{2+}$ channels [13], activation of $Ca^{2+}$ regulatory mechanisms or both processes acting simultaneously.

Glucose modulates electrical activity by increasing the duration of the 'bursts' in the range 7–20 mM [14]. This effect has a correlate in terms of intracellular $Ca^{2+}$ as shown in fig.4. In the presence of 11 mM glucose the $[Ca^{2+}]_i$ underwent oscillations as described above. Raising glucose concentration to 16.7 mM resulted in a transitory phase of elevated $[Ca^{2+}]_i$ lasting 3–4 min; resumption of oscillatory $[Ca^{2+}]_i$ activity occurred after a silent period with slightly depressed $Ca^{2+}$ levels. The most apparent difference between the oscillations in 11 and 16.7 mM glucose was its longer duration at the latter concentration (the average times that the islet spent at high plateau $Ca^{2+}$ levels in 11 and 16.7 mM glucose were 32% and 57%, respectively), with little or no change in amplitude.

## 4. DISCUSSION

The main finding of this paper involves the detection of high frequency $[Ca^{2+}]_i$ oscillations with brief rising times ('fast' oscillations) in single mouse islets of Langerhans. In addition, slowly developing cyclic $[Ca^{2+}]_i$ transients occurring at a much lower frequency ('slow' oscillations) were detected.

Several lines of evidence support the idea that 'fast' calcium oscillations are the direct consequence of $\beta$-cell bursting electrical activity. Thus: (i) these oscillations have a duration (3–11 s) and frequency (2–5 $min^{-1}$) in 11 mM glucose of the same order of magnitude as those of the glucose-induced bursts of electrical activity (ca. 6 s and 2–4 $min^{-1}$, respectively [7,10]); (ii) the brief rising time of the $[Ca^{2+}]_i$ transients (1–2 s) is consistent with the high instantaneous spike frequency measured at the beginning of each burst (ca. 7 $s^{-1}$ [14]); (iii) $[Ca^{2+}]_i$ oscillations appear at a glucose concentration somewhere within the range 5.5–11.1 mM, which includes the glucose threshold for activation of electrical activity (ca. 7 mM [10]); (iv) glucose increases the duration of the $[Ca^{2+}]_i$ oscillations in much the same way as it augments the duration of the bursts of membrane potential; and finally, (v) the biphasic changes produced by increasing glucose concentration from non-stimulatory to stimulatory levels, i.e. a transient $[Ca^{2+}]_i$ rise followed by the appearance of oscillations, are paralleled by biphasic changes in bursting electrical activity [11].

Neither 'fast' $[Ca^{2+}]_i$ oscillations nor bursting electrical activity have been reported so far in isolated $\beta$-cells [6,15]. A possible explanation is that intercellular coupling is a requisite step for $\beta$-cells to undergo the cyclic changes in membrane potential found in their original environment. The fact that regular 'fast' $[Ca^{2+}]_i$ oscillations can be recorded at all from an intact islet strongly suggests the existence of extensive networks of electrically coupled cells. Further evidence for this concept comes from findings that both $K^+$ and $Ca^{2+}$ concentrations in the extracellular space of the islet oscillate in phase with $\beta$-cell membrane potential [16]. Moreover, electrical synchrony within islet cell pairs has been demonstrated by double-microelectrode intracellular recording techniques [17]. Thus, the present results provide a framework for the interpretation of islet properties, such as pulsatile insulin secretion [18], that require its functioning as a 'syncytium'.

In addition to the 'fast' $[Ca^{2+}]_i$ oscillations discussed

above, a second type of oscillation ('slow') was found. Are these two types of oscillations independent processes or do they represent the same underlying phenomenon? On the one hand, it is conceivable that the 'slow' oscillations might be due to the averaging of slightly out-of-phase 'fast' $[Ca^{2+}]_i$ waves originating from uncoupled cell territories. This would occur only if: (1) the duration of the electrical bursts of the individual cells were small compared to the period of the oscillation; and (2) the frequency of these bursts were of the same order of magnitude as the frequency of the $[Ca^{2+}]_i$ oscillation, i.e. 0.2–1 $min^{-1}$. Burst frequencies in this range are, however, extremely rare [7]. Moreover, we found the 'slow' oscillations to be insensitive to glucose in the range 11–22 mM. Therefore, we envisage the 'fast' and 'slow' $[Ca^{2+}]_i$ oscillations to be processes that represent different underlying phenomena. It is noteworthy that our 'slow' oscillations resemble those recently found in isolated pancreatic $\beta$-cells [6]. Although its origin is unknown, it is tempting to correlate the 'slow' oscillations with previously reported cyclic variations in the intensity of $\beta$-cell electrical activity [19]. These secondary oscillations were found to occasionally superimpose on the bursts of membrane potential. Clearly, further studies are necessary to elucidate the nature of the 'slow' oscillations.

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