Distinct effects of various amino acids on $^{45}\text{Ca}^{2+}$ fluxes in rat pancreatic islets

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The effects of three types of amino acids on $^{45}\text{Ca}^{2+}$ fluxes in rat pancreatic islets have been compared. Alanine, a non-insulinotropic neutral amino acid, transported with Na^+ , increased $^{45}\text{Ca}^{2+}$ efflux in the presence or in the absence of extracellular Ca^{2+} , but not in the absence of Na^+ . Its effects in Na^+ -solutions were practically abolished by 7 mM-glucose. Alanine slightly stimulated $^{45}\text{Ca}^{2+}$ influx (5 min uptake) only when Na^+ was present. Two insulinotropic cationic amino acids (arginine and lysine) triggered similar changes in $^{45}\text{Ca}^{2+}$ efflux. They accelerated the efflux in the presence of Ca^{2+} and inhibited the efflux in a Ca^{2+} -free medium, whether glucose was present or not. In an Na^+ -free Ca^{2+} -medium, arginine and lysine markedly accelerated $^{45}\text{Ca}^{2+}$ efflux, but this effect was suppressed by 7 mM-glucose. Arginine stimulated $^{45}\text{Ca}^{2+}$ influx irrespective of the presence or absence of glucose and Na^+ . Leucine, a neutral insulinotropic amino acid well metabolized by islet cells, inhibited $^{45}\text{Ca}^{2+}$ efflux from the islets in a Ca^{2+} -free medium; this effect was potentiated by glutamine. In the presence of Ca^{2+} and Na^+ , leucine was ineffective alone, but triggered a marked increase in $^{45}\text{Ca}^{2+}$ efflux when combined with glutamine. In an Na^+ -free Ca^{2+} -medium, leucine accelerated $^{45}\text{Ca}^{2+}$ efflux to the same extent with or without glutamine. Leucine also stimulated $^{45}\text{Ca}^{2+}$ influx in the presence or in the absence of Na^+ , but its effects were potentiated by glutamine only in the presence of Na^+ . The results show that amino acids of various types cause distinct changes in $^{45}\text{Ca}^{2+}$ fluxes in pancreatic islets. Certain of these changes involve an Na^+ -mediated mobilization of cellular Ca^{2+} from sequestering sites where glucose appears to exert an opposite effect.

Amino acids stimulate insulin release *in vivo* (Floyd *et al.*, 1966) and *in vitro* (Milner & Hales, 1967; Sussman *et al.*, 1967). Although the involvement of Ca^{2+} in the stimulation of insulin release by glucose and the changes in $^{45}\text{Ca}^{2+}$ fluxes brought about by the sugar in islet cells have been extensively characterized (Wollheim & Sharp, 1981), few studies have addressed the role of Ca^{2+} in the response to amino acids. Extracellular Ca^{2+} is required for leucine (Milner & Hales, 1967; Malaisse-Lagae *et al.*, 1971) or arginine (Gerich *et al.*, 1974) to stimulate insulin release and the effect of these amino acids is suppressed when Ca^{2+} channels are blocked by cobalt (Henquin & Lambert, 1975), verapamil (Leclercq-Meyer *et al.*, 1978) or D600 (Henquin *et al.*, 1982). Leucine and arginine both increase $^{45}\text{Ca}^{2+}$ net uptake by islet cells

(Malaisse-Lagae *et al.*, 1971; Hellman *et al.*, 1977; Malaisse *et al.*, 1981) and their stimulatory effect is blocked by D600 (Henquin *et al.*, 1982). Finally, leucine, like glucose, accelerates $^{45}\text{Ca}^{2+}$ efflux from isolated islets perfused in the presence of Ca^{2+} , but decreases the efflux rate in the absence of extracellular Ca^{2+} (Malaisse *et al.*, 1980).

In the present study, we have compared the changes in $^{45}\text{Ca}^{2+}$ fluxes induced by amino acids of various types in isolated rat islets, and evaluated the influence of glucose on these changes as well as their dependence on extracellular Ca^{2+} and Na^+ . The amino acids tested were: alanine, which is transported in islet cells with Na^+ (Hellman *et al.*, 1971b), but is barely oxidized in the absence of glucose (Hellman *et al.*, 1971a) and does not induce insulin release; arginine, which is transported in islet cells by a system specific for cationic amino acids (Hellman *et al.*, 1971b) but is not metabolized (Hellman *et al.*, 1971a); leucine, the transport of

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which is only partially Na^+ -dependent (Hellman *et al.*, 1971b) and which is actively metabolized by islet cells (Hellman *et al.*, 1971a; Malaisse *et al.*, 1980). A preliminary account of some of the results has been published (Charles & Henquin, 1982).

Materials and methods

All experiments were made with islets isolated by collagenase digestion of the pancreas of fed male Wistar rats. The medium used was a Krebs–Ringer bicarbonate buffer, pH 7.4, gassed with O_2/CO_2 (47:3), with the following ionic composition (mM): NaCl , 118; KCl , 4.8; CaCl_2 , 2.5; MgSO_4 , 1.2; KH_2PO_4 , 1.2; NaHCO_3 , 24. It was supplemented with 5 mg of bovine serum albumin/ml. When CaCl_2 was omitted from the medium, the concentration of NaCl was increased to 122 mM and the total Ca^{2+} concentration, measured by atomic-absorption spectroscopy, was less than $20 \mu\text{M}$. Nominally Na^+ -free solutions were prepared by substituting choline chloride and choline bicarbonate for Na^+ salts; these solutions were supplemented with $10 \mu\text{M}$ -atropine to prevent any cholinergic effect of choline. Glucose or the amino acids studied were added to the medium without correction for osmolarity, but, when necessary, the pH of the medium was adjusted back to 7.4 after addition of the test substance. Stability of the pH was systematically checked at the end of the experiments. To prevent any change in pH in the small volumes of solutions used for measurements of $^{45}\text{Ca}^{2+}$ uptake, the medium was also supplemented with 10 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] and neutralized by 4–4.5 mM-NaOH. If the solutions were Na^+ -free, KOH was used instead of NaOH, and the concentration of KCl was appropriately decreased to maintain a final concentration of 6 mM- K^+ .

The perfusion system used to monitor the efflux of $^{45}\text{Ca}^{2+}$ from preloaded islets was the same as that used to follow the efflux of $^{42}\text{K}^+$ or $^{86}\text{Rb}^+$ (Henquin, 1978, 1980). Batches of 40 islets were first incubated at 37°C for 90 min in 0.3 ml of medium containing 15 mM-glucose and a trace amount of $^{45}\text{CaCl}_2$ (0.18 mCi/ml). After being washed three times with non-radioactive medium, the islets were transferred to the perfusion chambers. $^{45}\text{Ca}^{2+}$ in the effluent fractions (2.2 ml) collected at 2 min intervals and remaining in the islets at the end of the experiments was counted in a liquid-scintillation spectrometer. For each collection interval, the fractional efflux of $^{45}\text{Ca}^{2+}$ was calculated ($^{45}\text{Ca}^{2+}$ released during time interval/ $^{45}\text{Ca}^{2+}$ remaining in tissue during that time interval). $^{45}\text{Ca}^{2+}$ uptake by islet cells was measured as described previously (Henquin & Lambert, 1975), using $[6,6\text{'-}^3\text{H}]\text{sucrose}$ as marker of the extracellular space.

All amino acids were of the L-form and were obtained from the following sources: alanine, lysine hydrochloride and leucine from Merck A.G., Darmstadt, Germany; arginine hydrochloride and glutamine from Aldrich Europe, Beerse, Belgium.

Results

The influence of the ionic composition of the medium on the rate of $^{45}\text{Ca}^{2+}$ efflux from control islets not stimulated by amino acids is detailed in Table 1. The time course of this efflux in the absence of glucose and in the presence of 7 mM-glucose is illustrated by Figs. 1 and 2 respectively. Whatever the composition of the medium, the efflux rate slightly decreased with time during the 90 min of experiment. When CaCl_2 was omitted from the perfusion medium, the efflux rate was increased in the absence, but not in the presence, of glucose. This observation is in keeping with the well established inhibitory effect of glucose on $^{45}\text{Ca}^{2+}$ efflux from islets perfused in the absence of extracellular Ca^{2+} (Wollheim & Sharp, 1981). The rate of $^{45}\text{Ca}^{2+}$ efflux was slightly decreased when choline salts were substituted for Na^+ salts in the absence or in the presence of glucose. The lowest rate of efflux was recorded when both Ca^{2+} and Na^+ were omitted, but the residual efflux, not influenced by the presence of glucose, still amounted to more than half the efflux rate measured under control conditions (Table 1).

Effects of alanine on $^{45}\text{Ca}^{2+}$ efflux

Addition of 20 mM-alanine to a control medium not containing glucose accelerated $^{45}\text{Ca}^{2+}$ efflux from

Table 1. Effects of Ca^{2+} , Na^+ and glucose on the fractional efflux rate of $^{45}\text{Ca}^{2+}$ from perfused rat islets

The composition of the perfusion medium remained the same during the whole of each experiment. The time course of $^{45}\text{Ca}^{2+}$ efflux is shown in Figs. 1 and 2 and the Table gives the values 40 and 80 min after the start of the efflux. Values are means $\pm$ S.E.M. of five experiments. *Significantly different (P at least <0.01 by unpaired t test) from the control value in the presence of Ca^{2+} and Na^+ .

Composition of medium (mM)			Fractional efflux rate of $^{45}\text{Ca}^{2+}$	
Glucose	Ca^{2+}	Na^+	40 min	80 min
0	2.5	142	0.0080 ± 0.0006	0.0063 ± 0.0003
0	0	146	$0.0113 \pm 0.0005^*$	$0.0097 \pm 0.0006^*$
0	2.5	0	0.0066 ± 0.0004	$0.0048 \pm 0.0003^*$
0	0	0	$0.0052 \pm 0.0002^*$	$0.0035 \pm 0.0002^*$
7	2.5	142	0.0085 ± 0.0002	0.0053 ± 0.0003
7	0	146	0.0081 ± 0.0006	0.0050 ± 0.0003
7	2.5	0	$0.0068 \pm 0.0004^*$	0.0044 ± 0.0002
7	0	0	$0.0046 \pm 0.0002^*$	$0.0033 \pm 0.0003^*$

Fig. 1. *Effects of alanine on $^{45}\text{Ca}^{2+}$ efflux from rat islets perfused in the absence of glucose*
The ionic composition of the medium is shown in each panel. L-Alanine (20 mM) was added over the period indicated (O). Controls without addition of alanine are shown by the filled circles. Values are means $\pm$ s.e.m. of five control experiments and four experiments with alanine.

Fig. 2. *Effects of alanine on $^{45}\text{Ca}^{2+}$ efflux from rat islets perfused in the presence of 7 mM-glucose*
The ionic composition of the medium is shown in each panel. L-Alanine (10 mM) was added over the period indicated (O). Controls without addition of alanine are shown by the filled circles. Values are means $\pm$ s.e.m. of five control experiments and four experiments with alanine.

the islets (Fig. 1a). This increase started only after 4–6 min and became stable after about 10–12 min. Upon removal of alanine, the efflux rate exhibited a small, transient, but consistent further increase, before decreasing to control values. In the absence of Ca^{2+} , alanine still increased $^{45}\text{Ca}^{2+}$ efflux (Fig. 1b). Again this effect was progressive and not immediately reversible upon withdrawal of the amino acid. By contrast, in the absence of Na^+ , alanine failed to affect $^{45}\text{Ca}^{2+}$ efflux from the islets, whether Ca^{2+} was or was not present in the medium (Figs. 1c and 1d).

If the control medium was supplemented with 7 mM-glucose, a small short-lived increase in $^{45}\text{Ca}^{2+}$ efflux occurred upon addition and removal of 10 mM-alanine (Fig. 2a). When either Ca^{2+} or Na^+ or both were omitted from the medium containing glucose, alanine was without effect on $^{45}\text{Ca}^{2+}$ efflux from the islets (Figs. 2b, 2c and 2d). Similar observations were made with a higher (20 mM) concentration of alanine (results not shown).

Effects of arginine and lysine on $^{45}\text{Ca}^{2+}$ efflux

Addition of 20 mM-arginine to a control medium not containing glucose accelerated $^{45}\text{Ca}^{2+}$ efflux from the islets (Fig. 3a). This increase was rapid, but lasted only 14–16 min. However, if the medium did not contain Na^+ , the stimulation of $^{45}\text{Ca}^{2+}$ efflux was

more marked and lasted until withdrawal of arginine, even if its magnitude diminished after 20 min (Fig. 3c). Under both conditions, removal of the amino acid was followed by a decrease of the efflux rate of $^{45}\text{Ca}^{2+}$ below control values. In the absence of Ca^{2+} , arginine caused a delayed and progressive inhibition of $^{45}\text{Ca}^{2+}$ efflux, whereas withdrawal of arginine was accompanied by a rapid increase to control values (Fig. 3b). In a Ca^{2+} - and Na^+ -free medium, arginine had no effect on $^{45}\text{Ca}^{2+}$ efflux (Fig. 3d). Essentially similar results were obtained when lysine was tested instead of arginine (Figs. 3a–3d).

If the control medium was supplemented with 7 mM-glucose, 10 mM-arginine or 10 mM-lysine induced a rapid and sustained increase in $^{45}\text{Ca}^{2+}$ efflux (Fig. 4a), which was practically abolished by omission of Na^+ (Fig. 4c). Both cationic amino acids caused a small but sustained and reversible decrease in the efflux rate when Ca^{2+} was absent from the glucose containing medium (Fig. 4b). This inhibitory effect was strongly reduced if both Ca^{2+} and Na^+ were omitted (Fig. 4d). Similar results were obtained when arginine or lysine was tested at a higher (20 mM) concentration (results not shown).

Effects of leucine on $^{45}\text{Ca}^{2+}$ efflux

In a control medium not containing glucose, the only significant change in $^{45}\text{Ca}^{2+}$ efflux produced by

Fig. 3. Effects of arginine and lysine on $^{45}\text{Ca}^{2+}$ efflux from rat islets perfused in the absence of glucose. The ionic composition of the medium is shown in each panel. L-Arginine (●) or L-lysine (○) (each at 20 mM) was added over the period indicated. Controls without addition of amino acid are shown by the broken line. Values are means $\pm$ S.E.M. of four experiments.

Fig. 4. Effects of arginine and lysine on $^{45}\text{Ca}^{2+}$ efflux from rat islets perfused in the presence of 7 mM-glucose. The ionic composition of the medium is shown in each panel. L-Arginine (●) or L-lysine (○) (each at 10 mM) was added over the period indicated. Controls without addition of amino acid are shown by the broken line. Values are means $\pm$ S.E.M. of four experiments.

20 mM-leucine was a delayed and progressive inhibition that was reversible after withdrawal of the amino acid (Fig. 5a). When the medium did not contain Ca^{2+} , leucine caused a faster decrease in the efflux rate. This effect was marked and sustained in the presence of Na^+ (Fig. 5b), but much smaller and transient if Na^+ had also been omitted (Fig. 5d). Under both conditions, removal of leucine was followed by an increase in $^{45}\text{Ca}^{2+}$ efflux, commensurate with the decrease produced by the amino acid. In the absence of Na^+ , but in the presence of Ca^{2+} , leucine transiently stimulated $^{45}\text{Ca}^{2+}$ efflux from the islets (Fig. 5c).

When the effects of leucine were evaluated in media supplemented with 7 mM-glucose, no significant changes in $^{45}\text{Ca}^{2+}$ efflux were observed (results not shown).

Glutamine is known to potentiate the secretory effects of leucine in islet cells (Sener *et al.*, 1981). Glutamine (10 mM) alone had no significant effect on the rate of $^{45}\text{Ca}^{2+}$ efflux, whatever the ionic composition of the medium. In its presence, however, leucine triggered a biphasic increase in $^{45}\text{Ca}^{2+}$ efflux from the islets perfused with a medium containing Ca^{2+} and Na^+ (Fig. 5a). Glutamine also potentiated the inhibition of efflux produced by leucine in the absence of Ca^{2+} (Fig. 5b). By contrast,

the effects of leucine on $^{45}\text{Ca}^{2+}$ efflux were barely affected by glutamine in Na^+ -free solutions (Figs. 5c and 5d).

Effects of alanine, arginine and leucine on $^{45}\text{Ca}^{2+}$ influx

To evaluate Ca^{2+} influx in islet cells, $^{45}\text{Ca}^{2+}$ uptake was measured during 5 min-incubations. The tracer was added together with the amino acid to estimate the initial effect, or 20 min after addition of the amino acid, to determine whether the effect was sustained.

Addition of alanine, arginine or leucine to a normal glucose-free medium stimulated $^{45}\text{Ca}^{2+}$ uptake in islet cells (Table 2). The effect of leucine was markedly potentiated ($P < 0.001$) in the presence of glutamine. The acceleration of uptake was still present 20 min after addition of alanine or arginine, although the effect of arginine was clearly smaller than during the initial 5 min of stimulation. On the other hand, the effect of leucine was no longer significant, unless glutamine was also present (Table 2).

When Na^+ was omitted from the incubation medium, basal $^{45}\text{Ca}^{2+}$ uptake measured in the absence of amino acid was increased ($P < 0.001$). Addition of alanine was without effect. Arginine and

Fig. 5. Effects of leucine on $^{45}\text{Ca}^{2+}$ efflux from rat islets perfused in the absence of glucose. The ionic composition of the medium is shown in each panel. L-Leucine (20 mM) was added over the period indicated. In one series of experiments, 10 mM-glutamine was present throughout (○). Controls without addition of amino acid are shown by the broken line. Values are means $\pm$ S.E.M. of four experiments.

Table 2. Effects of amino acids on $^{45}\text{Ca}^{2+}$ uptake by incubated rat islets

After preliminary incubation for 30 min in the absence of glucose and amino acid, batches of five islets were transferred into 50 μl of medium layered on silicone oil. This medium contained 142 mM- Na^+ (normal medium) or 142 mM-choline $^+$ (Na^+ -free medium) without glucose or with 7 mM-glucose. After 20 min at 37°C, 50 μl of prewarmed medium containing 2.5 mM- $^{45}\text{CaCl}_2$ (sp. radioactivity 16 Ci/mol) and 0.5 mM-[6,6'- ^3H]sucrose (sp. radioactivity 0.1 Ci/mmol) were added to the tubes. After 5 min, the islets were separated from the radioactive medium by centrifugation through the oil. To measure $^{45}\text{Ca}^{2+}$ uptake during the first 5 min of stimulation (0–5 min) by the amino acids, these amino acids were added at the same time as the radioactive tracers. When tested, however, glutamine was present from the start, and thus before addition of leucine. To measure $^{45}\text{Ca}^{2+}$ uptake after 20 min of stimulation (20–25 min) by the amino acids, these amino acids were added to the medium used for the initial 20 min incubation period, preceding addition of the radioactive tracers. Each experimental condition was tested in at least three experiments. Values are means $\pm$ S.E.M. for the numbers of batches of islets given in parentheses. Significance levels: * $P < 0.01$; ** $P < 0.001$, versus controls without amino acid (unpaired t test).

Composition of medium (mM)		$^{45}\text{Ca}^{2+}$ uptake (pmol/islet per 5 min)		
Glucose	Amino acid	Normal medium		Na^+ -free medium
		0–5 min	20–25 min	0–5 min
0	—	9.1 ± 0.4 (22)	9.4 ± 0.4 (15)	12.7 ± 0.4 (21)
0	Alanine (20)	12.1 ± 0.4 (22)**	11.8 ± 0.6 (15)*	13.1 ± 0.4 (21)
0	Arginine (20)	14.2 ± 0.6 (22)**	11.2 ± 0.4 (15)*	16.8 ± 0.4 (21)**
0	Leucine (20)	14.4 ± 0.5 (22)**	10.6 ± 0.5 (15)	18.0 ± 0.5 (21)**
0	Leucine (20) + glutamine (10)	19.0 ± 0.7 (22)**	15.5 ± 0.6 (15)**	18.6 ± 0.5 (21)**
7	—	12.3 ± 0.4 (16)	—	15.3 ± 0.4 (16)
7	Arginine (10)	16.8 ± 0.4 (16)**	—	19.0 ± 0.5 (16)**

leucine stimulated $^{45}\text{Ca}^{2+}$ uptake, but the effect of leucine was no longer potentiated by glutamine.

Whether the medium contained Na^+ or not, $^{45}\text{Ca}^{2+}$ uptake was increased by 7 mM-glucose and further stimulated by arginine (Table 2).

Discussion

It was not the scope of this study to characterize and to quantify the various components of $^{45}\text{Ca}^{2+}$ fluxes in islet cells, but certain observations made under control conditions (i.e. in the absence of amino acids) deserve comment. The present results confirm previous studies showing that, in islet cells, the efflux of $^{45}\text{Ca}^{2+}$ is markedly influenced by extracellular Ca^{2+} and Na^+ and could involve $\text{Ca}^{2+}/\text{Ca}^{2+}$ - and $\text{Na}^+/\text{Ca}^{2+}$ -exchange mechanisms (Frankel *et al.*, 1978; Henquin, 1979; Hellman *et al.*, 1980; Herchuelz *et al.*, 1980; Siegel *et al.*, 1980). The nature of the large residual efflux persisting in the absence of Na^+ and Ca^{2+} remains to be established, however. It is also obvious that the changes in $^{45}\text{Ca}^{2+}$ influx (5 min uptake) and $^{45}\text{Ca}^{2+}$ efflux are not necessarily parallel. In the absence of Na^+ , the efflux rate was slightly decreased, likely because of the suppression of an $\text{Na}^+/\text{Ca}^{2+}$ -exchange, but $^{45}\text{Ca}^{2+}$ influx was increased. Furthermore, 7 mM-glucose increased $^{45}\text{Ca}^{2+}$ influx in the presence and in the absence of Na^+ , but did not significantly change the efflux rate.

At first sight the amino acids studied may seem to produce similar effects on Ca^{2+} handling by islet cells. They all accelerate $^{45}\text{Ca}^{2+}$ efflux and stimulate $^{45}\text{Ca}^{2+}$ influx when the medium contains both Ca^{2+} and Na^+ . This latter property is probably due to their ability to depolarize the B-cell membrane (Henquin & Meissner, 1981; Meissner & Henquin, 1983) and to activate voltage-dependent Ca^{2+} channels (Henquin *et al.*, 1982). The differences, however, make it more instructive to discuss the effects of the three types of amino acids separately. Though chemically similar, they finally prove to be functionally very different substances.

Effects of alanine

Several characteristics of the changes in $^{45}\text{Ca}^{2+}$ fluxes brought about by alanine make this amino acid clearly distinguishable from leucine and from cationic amino acids: its stimulation of $^{45}\text{Ca}^{2+}$ influx and efflux shows an absolute requirement for extracellular Na^+ and its stimulation of $^{45}\text{Ca}^{2+}$ efflux persists in the absence of extracellular Ca^{2+} .

The abolition of the effects of alanine in an Na^+ -free medium can be explained by the requirement of Na^+ for alanine transport in B-cells (Hellman *et al.*, 1971b). It is not established whether the depolarization of B-cells by alanine (Henquin & Meissner, 1981) is suppressed in the absence of Na^+ ,

as is the depolarization of pancreatic acinar cells (Iwatsuki & Petersen, 1980). However, in contrast with leucine, which decreases K^+ -permeability of the B-cell membrane (Henquin & Meissner, 1981), alanine causes a large efflux of $^{86}\text{Rb}^+$ from islet cells, and this effect is inhibited by omission of Na^+ (J. C. Henquin, unpublished work).

The stimulation of $^{45}\text{Ca}^{2+}$ efflux by alanine persists when Ca^{2+} is omitted from the extracellular medium and thus probably results from a displacement of $^{45}\text{Ca}^{2+}$ from intracellular sequestering sites. An acceleration of $^{45}\text{Ca}^{2+}$ efflux from islets perfused with a Ca^{2+} -free medium has been observed before, when the intracellular concentration of Na^+ is increased by inhibiting the Na^+ pump with ouabain or a K^+ -free solution (Herchuelz & Malaisse, 1980a; Siegel *et al.*, 1980; Hellman *et al.*, 1982) or by activating Na^+ channels with veratridine (Herchuelz & Malaisse, 1980a). It thus seems reasonable to propose that Na^+ co-transported with alanine mobilizes cellular $^{45}\text{Ca}^{2+}$. In contrast with the rapid effects of alanine on the membrane potential of B-cells (Henquin & Meissner, 1981; Meissner & Henquin, 1983), on the efflux of $^{86}\text{Rb}^+$ (J. C. Henquin, unpublished work) and on $^{45}\text{Ca}^{2+}$ uptake, the acceleration of $^{45}\text{Ca}^{2+}$ efflux is slightly delayed. It is possible that a certain time is needed for intracellular Na^+ to reach a concentration sufficient to act on Ca^{2+} -sequestering sites. Alternatively, the increase in $^{45}\text{Ca}^{2+}$ efflux could be delayed by the inhibition of the $\text{Na}^+/\text{Ca}^{2+}$ exchange that is also expected to result from the rise in cellular $[\text{Na}^+]$. The reverse mechanism could also explain the 'rebound' increase in efflux seen upon withdrawal of alanine.

A third important characteristic of the effects of alanine on $^{45}\text{Ca}^{2+}$ efflux is their almost complete suppression by 7 mM-glucose, a concentration that does not affect the transport (Hellman *et al.*, 1971b) or depolarizing effect (Meissner & Henquin, 1983) of alanine, but increases its oxidation by mouse islet cells (Hellman *et al.*, 1971a). One could envisage first that glucose prevents $^{45}\text{Ca}^{2+}$ efflux by inhibiting the $\text{Na}^+/\text{Ca}^{2+}$ exchange (Herchuelz *et al.*, 1980; Siegel *et al.*, 1980). If such were the case, it would be difficult to explain why the expected rise in cytoplasmic $[\text{Ca}^{2+}]$ does not cause a sustained release of Ca^{2+} efflux. Secondly, the rise in cellular $[\text{Na}^+]$ could be less marked (e.g. because the Na^+ pump is activated). This is not supported by electrophysiological recordings showing that a marked hyperpolarization of the B-cell membrane, probably due to activation of an electrogenic pump, follows withdrawal of alanine (Meissner & Henquin, 1983). Furthermore, glucose also attenuated the activation of $^{45}\text{Ca}^{2+}$ efflux induced by ouabain or K^+ omission (Herchuelz & Malaisse, 1980a). Thirdly, glucose

could favour Ca^{2+} sequestration by cellular organelles and/or decrease the ability of Na^+ to mobilize it. A similar conclusion was reached in a recent study (Hellman *et al.*, 1982) showing that, in mouse islets, glucose inhibits insulin release induced by Na^+ -mobilization of intracellular Ca^{2+} .

Effects of arginine and lysine

Under all experimental conditions, the changes in $^{45}\text{Ca}^{2+}$ efflux produced by arginine and lysine were indistinguishable. These similarities reinforce our previous conclusion (Charles *et al.*, 1982) that cationic amino acids affect the B-cell function by a similar mechanism linked to their cationic nature and not to a specific group on the side chain. Although restricted to arginine, the following discussion therefore applies to lysine as well.

The effects of arginine on $^{45}\text{Ca}^{2+}$ efflux from the islets are markedly affected by changes in medium composition but are unlikely to result simply from alterations in the transport of the amino acid. Thus the effects of arginine on $^{86}\text{Rb}^+$ efflux from pre-loaded rat islets are essentially similar in the presence or in the absence of Ca^{2+} or Na^+ (Charles *et al.*, 1982), and glucose does not influence arginine transport in mouse islet cells (Hellman *et al.*, 1971b).

Arginine accelerates $^{45}\text{Ca}^{2+}$ efflux only in the presence of extracellular Ca^{2+} , when it also stimulates $^{45}\text{Ca}^{2+}$ entry in islet cells. This suggests the involvement of an exchange of sequestered $^{45}\text{Ca}^{2+}$ with non-radioactive entering $^{40}\text{Ca}^{2+}$. There is, however, no strict correlation between the increase in influx and efflux. In particular, in a Na^+ -free medium, arginine increases $^{45}\text{Ca}^{2+}$ influx to the same extent in the presence or in the absence of glucose, but $^{45}\text{Ca}^{2+}$ efflux is stimulated only in the absence of the sugar. Under these conditions no $\text{Na}^+/\text{Ca}^{2+}$ -exchange contributes to $^{45}\text{Ca}^{2+}$ efflux and it is unclear whether its acceleration reflects a true increase in net Ca^{2+} extrusion. It could merely be an increase in tracer extrusion because of a change in $^{45}\text{Ca}^{2+}$ specific radioactivity in the cytoplasm, brought about by the $^{40}\text{Ca}^{2+}/^{45}\text{Ca}^{2+}$ exchange in cellular organelles. The suppression of this effect in the presence of glucose could again be accounted for by an increased affinity of these sites for Ca^{2+} .

An inhibition of $^{45}\text{Ca}^{2+}$ efflux in the absence of extracellular Ca^{2+} is a characteristic of metabolized secretagogues like glucose (Wollheim & Sharp, 1981) or leucine (see below). A similar, albeit weaker, decrease is also produced by arginine, which, however, is not metabolized by mouse islet cells (Hellman *et al.*, 1971a). Since arginine has no effect on islet Ca^{2+} -dependent ATPases (Levin *et al.*, 1978), no mechanism is immediately apparent, but a stimulation by arginine of the metabolism of endogenous nutrients could be envisaged.

Effects of leucine

The changes in membrane permeability to K^+ and in membrane potential of B-cells induced by 10–20 mM-leucine are similar to those produced by a glucose concentration slightly above the threshold for stimulation of insulin release (Henquin & Meissner, 1981). The comparison holds true for the effects of this metabolized amino acid on $^{45}\text{Ca}^{2+}$ efflux. In a medium containing Na^+ , leucine alone had little effect in the presence of extracellular Ca^{2+} , but caused a marked inhibition in the absence of Ca^{2+} . These findings confirm previous results (Malaisse *et al.*, 1980) and are in keeping with the evidence that the inhibition of $^{45}\text{Ca}^{2+}$ efflux (in the absence of Ca^{2+}) is more sensitive to islet metabolic activity than the stimulation of $^{45}\text{Ca}^{2+}$ efflux (in the presence of Ca^{2+}) (Herchuelz & Malaisse, 1980b). It also seems probable that the stimulation of $^{45}\text{Ca}^{2+}$ efflux by leucine, in the presence of extracellular Ca^{2+} , involves a displacement of intracellular $^{45}\text{Ca}^{2+}$ by the entering non-radioactive $^{40}\text{Ca}^{2+}$. However, there appears to be no quantitative relationship between the rate of influx (5 min-uptake) and the rate of efflux. Thus, when leucine was used alone, $^{45}\text{Ca}^{2+}$ uptake was stimulated, but $^{45}\text{Ca}^{2+}$ efflux did not significantly change. Furthermore, in the presence of glutamine, leucine stimulated $^{45}\text{Ca}^{2+}$ uptake, but not $^{45}\text{Ca}^{2+}$ efflux, much more during the first 5 min than 20 min later. Finally, leucine accelerated $^{45}\text{Ca}^{2+}$ efflux in the absence of Na^+ , without stimulating $^{45}\text{Ca}^{2+}$ uptake more than in the presence of Na^+ (when no change in $^{45}\text{Ca}^{2+}$ efflux occurred). It is thus obvious that the rate of $^{45}\text{Ca}^{2+}$ efflux in the presence of extracellular Ca^{2+} is not always a reliable estimate of the rate of $^{45}\text{Ca}^{2+}$ influx.

Glutamine markedly potentiates the depolarization of the B-cell membrane (Meissner & Henquin, 1983) and the release of insulin (Sener *et al.*, 1981) triggered by leucine. It has also been reported that glutamine amplifies leucine-stimulation of $^{45}\text{Ca}^{2+}$ net uptake (90 min uptake) as well as the stimulatory and inhibitory effects of leucine on $^{45}\text{Ca}^{2+}$ efflux from islet cells (Malaisse *et al.*, 1981). Our results confirm these findings and further show that Na^+ substitution by choline suppresses the potentiation by glutamine of leucine-induced changes in $^{45}\text{Ca}^{2+}$ influx (5 min uptake) and efflux. The most plausible explanation is that glutamine requires Na^+ to enter islet cells as for other cells (Kilberg *et al.*, 1980; Shotwell *et al.*, 1981). This would be consistent with the observations that Na^+ omission prevents the increase in $^{86}\text{Rb}^+$ efflux triggered by glutamine (Charles *et al.*, 1982) and that glutamine oxidation by islet cells is impaired when the concentration of Na^+ is decreased to 24 mM (Sener *et al.*, 1982). On the other hand, the differences between the effects of alanine and

glutamine on $^{45}\text{Ca}^{2+}$ efflux do not necessarily invalidate this explanation. The two amino acids may enter islet cells at different rates and/or through systems showing various degrees of Na^{+} -dependency (Shotwell *et al.*, 1981). This is indirectly suggested by the much smaller effects of glutamine than alanine on the membrane potential of B-cells (Meissner & Henquin, 1983) and on $^{86}\text{Rb}^{+}$ efflux from islet cells (Charles *et al.*, 1982; J. C. Henquin, unpublished work). Furthermore, in the absence of glucose, glutamine (Sener *et al.*, 1982), but not alanine (Hellman *et al.*, 1971a), is actively metabolized by the islets.

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