A single mechanism for the stimulation of insulin release and $^{86}\text{Rb}^+$ efflux from rat islets by cationic amino acids

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The mechanisms by which cationic amino acids influence pancreatic B-cell function have been studied by monitoring simultaneously $^{86}\text{Rb}^+$ efflux and insulin release from perfused rat islets. The effects of two reference amino acids arginine and lysine were compared with those of closely related substances to define the structural requirements for recognition of these molecules as secretagogues. Arginine accelerated $^{86}\text{Rb}^+$ efflux and increased insulin release in the absence or in the presence of 7 mM-glucose. Its effects on efflux did not require the presence of extracellular Ca^{2+} or Na^+ , but its insulintropic effects were suppressed in a Ca^{2+} -free medium and inhibited in an Na^+ -free medium. Among arginine derivatives, only 2-amino-3-guanidinopropionic acid mimicked its effects on $^{86}\text{Rb}^+$ efflux and insulin release; citrulline, guanidinoacetic acid, 3-guanidinopropionic acid and guanidine were inactive. Norvaline and valine also increased $^{86}\text{Rb}^+$ efflux, but their effect required the presence of extracellular Na^+ ; they did not stimulate insulin release. Lysine as well as the shorter-chain cationic amino acids ornithine and 2,4-diaminobutyric acid accelerated $^{86}\text{Rb}^+$ efflux in a Ca^{2+} - and Na^+ -independent manner. Their stimulation of insulin release was suppressed by Ca^{2+} omission, but only partially inhibited in an Na^+ -free medium. The uncharged glutamine and norleucine increased the rate of $^{86}\text{Rb}^+$ efflux in the presence of glucose, only if extracellular Na^+ was present. Norleucine slightly increased release in a Ca^{2+} - and Na^+ -dependent manner. The effects of lysine on efflux and release were not mimicked by other related substances such as 1,5-diaminopentane and 6-aminohexanoic acid. The results suggest that the depolarizing effect of cationic amino acids is due to accumulation of these positively charged molecules in B-cells. This causes acceleration of the efflux of K^+ ($^{86}\text{Rb}^+$) and activation of the influx of Ca^{2+} (which triggers insulin release). The prerequisite for the stimulation of B-cells by this mechanism appears to be the presence of a positive charge on the side chain of the amino acid, rather than a specific group.

The ability of amino acids to stimulate insulin secretion was first recognized by studies *in vivo* (Floyd *et al.*, 1963). Despite numerous investigations (see Panten, 1975) on the transport, degradation and metabolic effects of amino acids in islet cells, the mechanisms by which they trigger insulin release are only now being unravelled.

It has been speculated that leucine and arginine, the most frequently studied amino acids, act on pancreatic B-cells by different mechanisms (Fajans *et al.*, 1972). This suggestion was mainly based on the observation that leucine- but not arginine-

induced insulin release in man is inhibited by diazoxide (Fajans *et al.*, 1967). From the characteristics of the release of insulin, it has also been inferred that the actions of arginine and glucose on B-cells are not only quantitatively, but also qualitatively, different (Fajans *et al.*, 1972; Levin *et al.*, 1972; Gerich *et al.*, 1974). For many years, however, the experimental evidence supporting these proposals has remained very limited. It was only known that, in contrast with glucose and leucine, arginine is practically not metabolized (Hellman *et al.*, 1971a) and does not increase the level of reduced nicotinamide nucleotides (Panten & Christians, 1973; Panten, 1975) in islet cells.

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Recent studies strongly suggest that the metabolic degradation of leucine by pancreatic B-cells is an integral part of stimulus–secretion coupling (Malaisse *et al.*, 1980). The evidence that acceleration of metabolic fluxes in islet cells also plays a central role in the insulinotropic effects of 4-methyl-2-oxopentanoate (a metabolite of leucine) and 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid (a non-metabolizable analogue of leucine) has been reviewed recently (Ashcroft, 1981; Malaisse *et al.*, 1981). In addition, leucine and its derivatives share with glucose the ability to depolarize the B-cell membrane by decreasing its permeability to K^+ and they induce an electrical activity similar to that produced by the sugar (Henquin & Meissner, 1981). By contrast, arginine increases the K^+ permeability of the B-cell membrane and its depolarizing effect has tentatively been ascribed to accumulation of that positively charged molecule in B-cells (Henquin & Meissner, 1981).

The present investigation, partially reported in abstract form (Charles *et al.*, 1981), is a 'structure–function' study of cationic amino acids. Its objective was to determine whether a single mode of action underlies the effects of structurally different cationic amino acids on pancreatic B-cells. We simultaneously monitored $^{86}Rb^+$ efflux and insulin release from perfused rat islets to establish whether there exists a link between the ability of these amino acids to change the K^+ permeability of the B-cell membrane (Henquin, 1980b) and to activate the releasing process. We further compared the effects of the reference amino acids arginine and lysine to those of closely related substances to define the structural requirements for recognition of these molecules as insulin secretagogues.

Materials and methods

All experiments were made with islets isolated by collagenase digestion of the pancreas of fed male Wistar rats and incubated or perfused in a bicarbonate-buffered medium (Henquin, 1980a). When $CaCl_2$ was omitted from the medium, the concentration of $NaCl$ was increased to maintain iso-osmolality and the total Ca^{2+} concentration, measured by atomic-absorption spectroscopy, was less than $30\mu M$. Na^+ -free solutions were prepared by substituting choline chloride and choline bicarbonate for Na^+ salts; these solutions were supplemented with $10\mu M$ -atropine to prevent any cholinergic effect of choline. The amino acids studied were added to the medium without correction for osmolality, 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.

The perfusion system used to monitor the efflux

of $^{86}Rb^+$ from preloaded islets has been described previously (Henquin, 1978). The same technique was used for the present experiments, with only slight modifications. Batches of 40 islets were first incubated at $37^\circ C$ for 2 h in 0.5 ml of medium containing 7 mM-glucose and 0.25–0.5 mM- $^{86}RbCl$ (sp. radioactivity 200–450 Ci/mol). After being washed three times with non-radioactive medium, they were transferred to the perfusion chambers. Effluent fractions (2.2 ml) were collected at 2 min intervals and a portion ($200\mu l$) was drawn for measurement of immunoreactive insulin with a double antibody assay using rat insulin as standard. The radioactivity present in each effluent fraction was counted and corrected for the portion drawn before calculation of the fractional efflux of $^{86}Rb^+$ ($^{86}Rb^+$ released during time interval/ $^{86}Rb^+$ remaining in tissue during that time interval).

The sources of test substances were as follows: leucine, lysine hydrochloride and guanidine chloride from Merck A.G., Darmstadt, Germany; norleucine, norvaline, 1,5-diaminopentane dihydrochloride, 6-aminohexanoic acid, 2,4-diaminobutyric acid hydrochloride and 2-amino-3-guanidinopropionic acid hydrochloride from Fluka A.G., Buchs, Switzerland; guanidinoacetic acid and 3-guanidinopropionic acid from Sigma Chemical Co., St. Louis, MO, U.S.A.; arginine hydrochloride, citrulline, ornithine hydrochloride, valine and glutamine from Aldrich Europe, Beerse, Belgium. All amino acids were of the L-form, except for DL-2,4-diaminobutyric acid.

Results are means $\pm$ S.E.M. The statistical significance of differences between experimental groups was assessed by Student's *t* test for unpaired data.

Results and discussion

Effects on $^{86}Rb^+$ efflux

(a) *Arginine and related substances.* In the absence of glucose, the rate of $^{86}Rb^+$ efflux from perfused islets declined slowly and regularly (Fig. 1). Addition of 20 mM-arginine (2-amino-5-guanidinopentanoic acid) to the medium resulted in a rapid increase in the efflux rate. This effect was completely reversible on removal of the amino acid (Fig. 1a). A derivative of arginine, lacking two methylene groups (2-amino-3-guanidinopropionic acid), also slightly increased $^{86}Rb^+$ efflux (Table 1). By contrast, substitution of the guanidine group of arginine by a non-ionized ureido group (citrulline; 2-amino-5-ureidopentanoic acid) was sufficient to make the molecule inactive. However, the effect cannot be ascribed to the guanidine group alone, since neither guanidine alone nor guanidinoacetic acid or 3-guanidinopropionic acid, which both lack an ionized α -amino group, increased $^{86}Rb^+$ efflux (Table 1). It was expected that the absence of the ionized guanidine group would produce an inactive

Table 1. *Effects of cationic amino acids and related substances on ⁸⁶Rb⁺ efflux and insulin release from rat islets*
After loading with ⁸⁶Rb⁺, the islets were perfused in the absence of glucose. After 40 min, test substances were added to the perfusion medium for 20 min. For each individual experiment the average fractional rate of ⁸⁶Rb⁺ efflux was calculated for the period 40–60 min of stimulation with the different test substances. These values are expressed as percentages of the average fractional efflux rate recorded, during the same period, in the absence of test substance. This absolute control value was 0.0243 ± 0.0006. The increase or decrease in insulin release (Δ) produced by the test substance was calculated as the total release during the test period (40–60 min), above or below a constant rate of secretion evaluated from the last 6 min (34–40 min) of the preceding control period. The total amount of insulin secreted by control islets during the reference period was 190 ± 18 pg/islet per 20 min. Results are means ± S.E.M. of *n* experiments. Significance levels: **P* < 0.05; ***P* < 0.005; ****P* < 0.001, versus controls without test substance.

Test substance (20 mM)	<i>n</i>	⁸⁶ Rb ⁺ efflux (% of controls)	Δ Insulin release (pg/islet per 20 min)
(a) None (control)	8	100 ± 2.7	−8 ± 8
(b) Arginine	4	121 ± 5.0**	+315 ± 30***
Citrulline	5	103 ± 3.8	+2 ± 44
2-Amino-3-guanidinopropionic acid	3	112 ± 3.6*	+12 ± 66
Guanidinoacetic acid	4	104 ± 8.0	−4 ± 42
3-Guanidinopropionic acid	4	102 ± 9.9	+10 ± 18
Guanidine	4	91 ± 3.2	−20 ± 16
Norvaline	5	118 ± 3.2**	+18 ± 18
Valine	4	109 ± 2.1*	−26 ± 22
(c) Lysine	4	117 ± 3.0**	+155 ± 28***
Ornithine	5	150 ± 7.0***	+132 ± 26***
Glutamine	5	96 ± 2.7	−34 ± 18
Norleucine	5	90 ± 1.8*	−18 ± 16
1,5-Diaminopentane	4	93 ± 4.6	−6 ± 34
6-Aminohexanoic acid	4	106 ± 6.0	−28 ± 20

Fig. 1. *Effects of various amino acids on ⁸⁶Rb⁺ efflux from perfused islets*
The experiments were carried out in the absence of glucose. The amino acids indicated in the Figure were added to the perfusion medium at the concentration of 20 mM, between 40 and 60 min. Control experiments with glucose-free medium alone are shown by the broken lines. Values are means ± S.E.M. of four (a and c) or five (b) experiments.

neutral molecule. Surprisingly, norvaline (2-amino-pentanoic acid) and its branched analogue valine accelerated ⁸⁶Rb⁺ efflux (Figs. 1b and 1c). Removal of norvaline, but not of valine, was paradoxically followed by a large rise in the rate of ⁸⁶Rb⁺ efflux (Fig. 1b).

In the presence of 7 mM-glucose, the rate of ⁸⁶Rb⁺ efflux was lower and more stable, at least in the control medium. In the absence of extracellular

Ca²⁺, the stability was less good and, in the absence of extracellular Na⁺, the rate of efflux continued to decrease slightly during the whole experiment (Fig. 2). Addition of arginine (10 mM) was followed by a rapid, marked and reversible increase in the efflux rate. This effect did not require the presence of extracellular Ca²⁺ or Na⁺. In the Na⁺-free medium, the efflux of ⁸⁶Rb⁺ fell below control values on removal of arginine (Fig. 2c). Among arginine

Fig. 2. Effects of arginine and lysine on $^{86}\text{Rb}^+$ efflux and insulin release from perfused islets

The experiments were carried out in the presence of 7 mM-glucose. Ca^{2+} was omitted from the medium in (b) and (b'); Na^+ was replaced by choline in (c) and (c'). As indicated, 10 mM-arginine (●) or -lysine (○) was added to the perfusion medium between 40 and 60 min. Control experiments without the addition of amino acid are shown by the broken lines. Values are means $\pm$ S.E.M. of five (a and a') or four (b, b', c and c') experiments.

Table 2. Changes in $^{86}\text{Rb}^+$ efflux produced by cationic amino acids and related substances in rat islets perfused without Na^+ and Ca^{2+}

The experiments were carried out in the presence of 7 mM-glucose, with a medium sometimes devoid of Ca^{2+} or Na^+ . The effect of the different test substances on $^{86}\text{Rb}^+$ efflux was calculated as in Table 1. For controls, the absolute values for the fractional rate of $^{86}\text{Rb}^+$ efflux were: 0.0148 ± 0.0002 in normal medium, 0.0144 ± 0.0002 in Ca^{2+} -free medium and 0.0118 ± 0.0002 in Na^+ -free medium. Results are means $\pm$ S.E.M. for the numbers of experiments shown in parentheses. Significance levels: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, versus controls without test substance.

Test substance (10 mM)	$^{86}\text{Rb}^+$ efflux (% of controls)		
	Normal medium	Ca^{2+} -free medium	Na^+ -free medium
(a) None (control)	100 ± 1.8 (6)	100 ± 1.5 (4)	100 ± 2.0 (4)
(b) Arginine	150 ± 1.4 (5)***	147 ± 9.2 (4)**	160 ± 1.9 (4)***
Citrulline	100 ± 3.5 (4)	—	—
2-Amino-3-guanidinopropionic acid	109 ± 1.1 (3)*	—	—
Guanidinoacetic acid	97 ± 0.8 (4)	—	—
3-Guanidinopropionic acid	101 ± 3.7 (4)	—	—
Guanidine	97 ± 4.3 (5)	—	—
Norvaline	136 ± 5.9 (5)***	130 ± 7.1 (3)**	110 ± 4.7 (4)
Valine	131 ± 4.0 (4)***	164 ± 6.7 (4)***	102 ± 2.8 (4)
(c) Lysine	145 ± 1.3 (5)***	144 ± 4.3 (4)***	152 ± 3.3 (4)***
Ornithine	159 ± 4.1 (6)***	143 ± 6.1 (4)***	170 ± 5.6 (4)***
2,4-Diaminobutyric acid	132 ± 4.1 (3)***	131 ± 1.7 (3)***	148 ± 4.1 (3)***
Glutamine	116 ± 2.2 (6)***	131 ± 2.7 (4)***	101 ± 1.7 (4)
Norleucine	114 ± 4.3 (7)*	121 ± 1.3 (3)***	103 ± 2.1 (4)
1,5-Diaminopentane	81 ± 3.9 (6)***	—	—
6-Aminohexanoic acid	87 ± 4.1 (6)*	—	—

derivatives, citrulline, guanidine, guanidinoacetic acid and 3-guanidinopropionic acid were without significant effect, whereas 2-amino-3-guanidinopropionic acid slightly increased the efflux rate (Table

2). Norvaline and valine again accelerated $^{86}\text{Rb}^+$ efflux; their effect was less marked than that of arginine, did not require extracellular Ca^{2+} but was suppressed in the absence of Na^+ (Table 2).

Table 3. *Changes in insulin release produced by cationic amino acids and related substances in rat islets perfused without Na⁺ and Ca²⁺*

The experiments, carried out in the presence of 7 mM-glucose, are the same as those presented in Table 2. The effect of the different test substances on insulin release was calculated as in Table 1. For controls, total amounts of insulin secreted during the reference period were: 1270 ± 116 pg/islet per 20 min in normal medium, 402 ± 54 pg/islet per 20 min in Ca²⁺-free medium and 570 ± 62 pg/islet per 20 min in Na⁺-free medium. Results are means $\pm$ S.E.M. for the numbers of experiments shown in parentheses. Significance levels: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, versus controls without test substance.

Test substance (10 mM)	Δ Insulin release (pg/islet per 20 min)		
	Normal medium	Ca ²⁺ -free medium	Na ⁺ -free medium
(a) None (control)	-20 ± 32 (6)	-25 ± 18 (4)	-124 ± 33 (4)
(b) Arginine	$+3260 \pm 196$ (5)***	-32 ± 14 (4)	$+204 \pm 80$ (4)*
Citrulline	$+8 \pm 78$ (4)	—	—
2-Amino-3-guanidinopropionic acid	$+764 \pm 146$ (3)**	—	—
Guanidinoacetic acid	-68 ± 32 (4)	—	—
3-Guanidinopropionic acid	-108 ± 90 (4)	—	—
Guanidine	-24 ± 96 (5)	—	—
Norvaline	-202 ± 138 (5)	$+20 \pm 14$ (3)	-48 ± 36 (4)
Valine	-528 ± 150 (4)*	$+2 \pm 6$ (4)	-45 ± 40 (4)
(c) Lysine	$+2020 \pm 306$ (5)***	-40 ± 37 (4)	$+296 \pm 96$ (4)**
Ornithine	$+1724 \pm 342$ (6)***	-46 ± 20 (4)	$+304 \pm 84$ (4)**
2,4-Diaminobutyric acid	$+984 \pm 208$ (3)***	-36 ± 58 (3)	$+650 \pm 123$ (3)**
Glutamine	$+208 \pm 154$ (6)	-34 ± 14 (4)	-94 ± 64 (4)
Norleucine	$+466 \pm 84$ (7)***	-28 ± 20 (3)	-102 ± 50 (4)
1,5-Diaminopentane	-18 ± 72 (6)	—	—
6-Aminohexanoic acid	-618 ± 126 (6)***	—	—

(b) *Lysine and related substances.* In the absence of glucose, 20 mM-lysine (2,6-diaminohexanoic acid) increased the rate of ⁸⁶Rb⁺ efflux from perfused islets (Fig. 1a). An even greater increase was observed on addition of ornithine (2,5-diaminopentanoic acid), whereas the uncharged glutamine (2,5-diamino-5-oxopentanoic acid) had no effect (Table 1). Norleucine (2-aminoheptanoic acid), which differs from lysine only in the absence of the NH₂ group in position-6, slightly decreased ⁸⁶Rb⁺ efflux (Fig. 1b). This decrease was, however, less marked than that produced by the branched amino acid leucine (Fig. 1c). On the other hand, the efflux rate was unaffected by 1,5-diaminopentane (the decarboxylation product of lysine) or by 6-aminoheptanoic acid, which lacks the α -amino group of lysine.

In the presence of 7 mM-glucose, 10 mM-lysine rapidly, markedly and reversibly increased the rate of ⁸⁶Rb⁺ efflux. This increase also occurred when Ca²⁺ or Na⁺ was omitted from the perfusion medium (Fig. 2). As previously observed with arginine, removal of lysine from the Na⁺-free medium was followed by a fall in the efflux rate below control values (Fig. 2c). A similar Ca²⁺- and Na⁺-independent acceleration of ⁸⁶Rb⁺ efflux was also produced by ornithine and by an even shorter cationic amino acid, 2,4-diaminobutyric acid (Table

2). Although uncharged, glutamine and norleucine brought about a slight increase in the rate of ⁸⁶Rb⁺ efflux; this effect persisted in the absence of extracellular Ca²⁺, but was suppressed by omission of Na⁺ (Table 2). On the other hand, 1,5-diaminopentane and 6-aminoheptanoic acid decreased ⁸⁶Rb⁺ efflux (Table 2); withdrawal of the former of these substances was followed by a rebound increase above control values (results not shown).

From all these experiments with arginine and lysine derivatives it appears that the ability to increase ⁸⁶Rb⁺ efflux from islet cells is a common property of quite different molecules, which all are cationic amino acids. This property is Na⁺- and Ca²⁺-independent, but is suppressed by small alterations of the molecule, which change its ionization state. This strongly suggests that the presence of a positive charge on the side chain, rather than the nature of the group responsible for the charge, is the prerequisite for an increase in the efflux rate. A further test of this hypothesis would be to change the proportion of molecules with an ionized basic group on the side chain. In practice this is not possible, however, because the pK values of these ionizable groups are too far away from the physiological pH. The interpretation of such experiments would also be complicated by the marked effect of extracellular pH on ⁸⁶Rb⁺ efflux from islet cells (Henquin, 1981).

At first glance, this hypothesis may seem inconsistent with the increase in $^{86}\text{Rb}^+$ efflux brought about by the neutral amino acids norvaline, valine, norleucine and glutamine. However, the Na^+ -dependency of the effect of these substances makes their property clearly distinguishable from that of cationic amino acids. The most plausible explanation is that they are transported in islet cells with Na^+ and that this influx of positive charges causes the efflux of K^+ . In other tissues, glutamine, norvaline and norleucine are transported by the Na^+ -requiring system A or ASC (Christensen, 1973). Valine, like leucine, usually prefers the Na^+ -independent system L, but it should be noted that in islet cells, leucine uptake is partially Na^+ -dependent (Hellman *et al.*, 1971b). This may indicate some participation of the system A for the transport of these branched-chain amino acids (Christensen, 1973). It is also pertinent to mention that, in the exocrine pancreas, the small depolarization produced by valine has been ascribed to an increase in membrane Na^+ conductance (Iwatsuki & Petersen, 1980).

Effects on insulin release

(a) *Arginine and related substances.* The insulinotropic effect of arginine in the absence of glucose has long been controversial (see Gerich *et al.*, 1974; Panten, 1975). Table 1 shows that in perfused rat islets 20 mM-arginine produced a weak but definite stimulation of insulin release. A similar effect was also observed with incubated rat islets (Henquin *et al.*, 1982). By contrast, none of the arginine derivatives increased insulin release in the absence of the sugar (Table 1).

In control conditions, 7 mM-glucose steadily stimulated insulin release; this effect was abolished in a Ca^{2+} -free medium and progressively faded out in an Na^+ -free medium (Fig. 2). Addition of 10 mM-arginine to the normal medium containing 7 mM-glucose triggered a rapid and large increase in insulin release, which stopped after withdrawal of the amino acid. Such a stimulation did not occur in the absence of extracellular Ca^{2+} and was markedly impaired, though not abolished, in the absence of extracellular Na^+ (Figs. 2b' and 2c'; Table 3). Only 2-amino-3-guanidinopropionic acid was able to mimic, less powerfully, the releasing effect of arginine. Guanidinoacetic acid, 3-guanidinopropionic acid, guanidine and norvaline had no significant effect. Glucose-induced insulin release was slightly inhibited by valine in the normal medium; this branched-chain amino acid was without effect in the absence of Ca^{2+} or Na^+ (Table 3).

The molecular requirements for the stimulation of insulin release by arginine have already been evaluated in a few previous studies. It was first reported that guanidine and guanidinoacetic acid were able to increase insulin release in the perfused

pancreas and this observation prompted the suggestion that the arginine molecule had to be converted into such products to trigger the releasing process (Alsever *et al.*, 1970). An acute intravenous injection of guanidine to rats increased plasma insulin levels, but this effect was inhibited by atropine (Aynsley-Green & Alberti, 1974). With isolated rat islets, guanidine proved ineffective in this and in another study (Marco *et al.*, 1976). The effect of guanidinoacetic acid could not be confirmed *in vivo* (Aynsley-Green & Alberti, 1974) or *in vitro* (Alberti & Whalley, 1973), but the cationic amino acids 2-amino-3-guanidinopropionic acid and 2-amino-4-guanidinobutyric acid increased insulin release in the presence of glucose (Alberti & Whalley, 1973). In one report (Sener *et al.*, 1981) norvaline was found to stimulate insulin release, but it proved inactive in this and two other studies (Alberti & Whalley, 1973; Schönborn *et al.*, 1975). Taken together our results and those of Alberti & Whalley (1973) show that the insulinotropic property of arginine is lost if the ionization state of the molecule is altered by removing one of its basic groups.

(b) *Lysine and related substances.* In a glucose-free medium, 20 mM-lysine stimulated insulin release. Among its derivatives, only ornithine exerted a similar effect (Table 1).

In the presence of 7 mM-glucose, 10 mM-lysine triggered a marked and reversible increase in insulin release. This effect was abolished in the absence of extracellular Ca^{2+} , but not totally suppressed by omission of Na^+ (Fig. 2). Insulin release was also augmented by the two other cationic amino acids, ornithine and 2,4-diaminobutyric acid; this increase had an absolute requirement for extracellular Ca^{2+} , but was only partially inhibited in the absence of Na^+ (Table 3). In agreement with the results of a recent study using incubated rat islets (Sener *et al.*, 1981), glutamine was without effect and norleucine increased insulin release (Table 3). Interestingly, the effect of norleucine was much weaker than that of related cationic amino acids and was completely suppressed in the absence of Na^+ . 1,5-Diaminopentane did not alter glucose-induced release, but its withdrawal from the perfusion medium was accompanied by an unexplained increase in secretion (results not shown). For an unknown reason, 6-aminohexanoic acid partially and reversibly inhibited insulin release induced by 7 mM-glucose (Table 3).

The complete suppression of the insulinotropic effect of all these cationic amino acids by omission of extracellular Ca^{2+} suggests that an influx of Ca^{2+} is involved in the stimulus-secretion coupling. This is in complete agreement with the blockade by compound D600 of arginine- or lysine-stimulated insulin release and Ca^{2+} uptake (Henquin *et al.*, 1982). By contrast, cationic amino acids remained

able to increase release in the absence of Na^+ , whereas the insulinotropic effect of glucose itself was diminishing. It is not clear whether their reduced effectiveness in the Na^+ -free medium is merely due to suppression of the potentiating effect of glucose, the metabolism of which is impaired in the absence of extracellular Na^+ (Hellman *et al.*, 1974), or to another mechanism. Additional studies are necessary to answer that question.

General discussion and conclusions

The ability of cationic amino acids to trigger an efflux of K^+ ($^{86}\text{Rb}^+$) is not restricted to islet cells. Their accumulation in muscle cells is also accompanied by a loss of cellular K^+ (Levinsky *et al.*, 1962; Arnauld & Lachance, 1980), which likely accounts for the rise in plasma $[\text{K}^+]$ that can be measured during intravenous infusion of arginine (Dicherman & Walker, 1964; Massara *et al.*, 1979). A similar efflux of K^+ also takes place during uptake of cationic amino acids by plant cells (Komor *et al.*, 1981).

Previous studies (Hellman *et al.*, 1971b; Christensen *et al.*, 1971; Cooperstein & Lazarow, 1977) have shown that mouse or toadfish islet cells markedly accumulate arginine, lysine and 4-amino-1-guanylpiperidine-4-carboxylic acid, a non-metabolizable analogue for the transport system of cationic amino acids. It thus seems reasonable to admit that pancreatic B-cells are equipped with such a system and that it is most likely Na^+ -independent as in other tissues (Heinz, 1972; Christensen, 1973).

The present study strongly suggests that the different cationic amino acids can be viewed as a single family of substances that stimulate B-cells by a single simple mechanism. The accumulation of these positively charged molecules in B-cells appears to be an electrogenic process; thus, arginine depolarizes the plasma membrane of mouse B-cells (Henquin & Meissner, 1981) and a similar effect has been observed recently with lysine and ornithine (J. C. Henquin & H. P. Meissner, unpublished work). The depolarization may, in turn, activate voltage-dependent Ca^{2+} -channels and the ensuing Ca^{2+} influx trigger insulin release (hence the suppression of release in the absence of Ca^{2+}). Simultaneously, the depolarization causes an efflux of K^+ ($^{86}\text{Rb}^+$) through voltage-dependent K^+ -channels, an effect that does not require the presence of Ca^{2+} to occur. The prerequisite to trigger these events is thus the presence of a positive charge on the side chain rather than a specific group.

The major characteristic of the stimulus-secretion coupling for these cationic amino acids is that, contrary to glucose or leucine and their analogues, it does not involve a decrease in the K^+ permeability of the B-cell membrane. As diazoxide inhibits B-cell

function by increasing the K^+ permeability of its plasma membrane (Henquin *et al.*, 1982), it is easy to understand why leucine- but not arginine-stimulated insulin release is inhibited by the drug, as originally observed *in vivo* (Fajans *et al.*, 1967) and recently confirmed *in vitro* (Henquin *et al.*, 1982). Finally, the simple mode of action proposed here for these cationic amino acids may account for their depolarizing effect in other tissues like the exocrine pancreas (Laugier & Petersen, 1981). It may explain their stimulation of glucagon release (Assan *et al.*, 1977) and of the secretory function of other gland cells equipped with voltage-dependent Ca^{2+} -channels.

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