Stimulation of insulin release by an organic calcium agonist

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Summary. The calcium-agonist 4-[2-(difluoromethoxy)phenyl]-1,4,5,7-tetrahydro-2-methyl-5-oxo-furo[3,4-b]pyridine-3-carboxylic acid ethylester provoked, in the 1.0–100 $\mu\text{mol/l}$ range, a dose-related increase of glucose-stimulated insulin release by rat pancreatic islets. A fixed concentration of the drug (50 $\mu\text{mol/l}$) caused a shift to the left of the sigmoidal curve relating insulin output to glucose concentration. The drug failed to affect insulin release evoked, in the absence of Ca^{2+} , by the combination of Ba^{2+} and theophylline. The enhancing action of the calcium-agonist upon insulin release was rapid and sus-

tained, and coincided with stimulation of both ^{45}Ca net uptake and ^{45}Ca efflux, the latter phenomenon being abolished in the absence of extracellular Ca^{2+} . It is concluded that the gating of Ca-channels, as presumably provoked by the calcium-agonist, simulates the stimulant action of glucose upon both Ca influx into and insulin release from the pancreatic islets.

Key words: Pancreatic islets, insulin release, calcium-agonist.

Recently, several compounds, which are chemically related to organic calcium-antagonists, were found to act as calcium-agonists, apparently causing the gating of calcium-channels and stimulation of calcium-dependent physiological processes [1–5]. In the present study, we have explored the effect of one of these agents, 4-[2-(difluoromethoxy)phenyl]-1,4,5,7-tetrahydro-2-methyl-5-oxo-furo[3,4-b]pyridine-3-carboxylic acid ethylester, upon calcium fluxes and insulin release in rat pancreatic islets.

Materials and methods

The drug 4-[2-(difluoromethoxy)phenyl]-1,4,5,7-tetrahydro-2-methyl-5-oxo-furo[3,4-b]pyridine-3-carboxylic acid ethylester (CGP 28392) was kindly provided by Dr. L. Best (Department of Pharmacology, University of London), and solubilized in dimethylsulphoxide at an initial concentration of 25 or 50 mmol/l. The same concentration of the solvent (not exceeding 2 $\mu\text{l/ml}$) was added to control incubation media. At this concentration, dimethylsulphoxide fails to affect islet function [6].

All experiments were conducted with islets isolated from the pancreas of fed albino rats [7]. The methods used to measure insulin release [8] and ^{45}Ca net uptake [9] in static incubations and insulin release [10] and ^{45}Ca fractional outflow rate [11] from prelabelled perifused islets were already described in the cited references. Briefly insulin release was measured by incubating for 90 min groups of eight islets each in 1.0 ml of bicarbonate-buffered media. The net uptake of ^{45}Ca was also measured in groups of eight islets over 90 min incubation, followed by repeated washes to remove extracellular ^{45}Ca . In the

perifusion experiments, the islets were prelabelled for 60 min in the presence of ^{45}Ca and D-glucose (16.7 mmol/l) and then placed in the perifusion chamber.

Statistical analyses

All results are expressed as mean $\pm$ SEM together with the number of individual observations and statistical significance of differences between control and test values, as assessed by Student's *t*-test.

Results

Secretory data

Glucose (2.8 to 16.7 mmol/l) caused a dose-related increase in insulin output (Table 1). At a concentration of 50 $\mu\text{mol/l}$, CGP 28392 augmented insulin output in the

Table 1. Effect of CGP 28392 upon glucose-induced insulin release

D-glucose (mmol/l)	Insulin output ($\mu\text{U}/90$ min per islet)		<i>p</i>
	Control	CGP 28392 (50 $\mu\text{mol/l}$)	
2.8	12.8 $\pm$ 1.5 (24)	15.1 $\pm$ 1.3 (24)	NS
5.6	23.7 $\pm$ 2.3 (24)	69.9 $\pm$ 4.9 (24)	<0.001
7.0	55.1 $\pm$ 3.7 (30)	100.5 $\pm$ 9.9 (30)	<0.001
11.1	158.6 $\pm$ 8.7 (35)	207.1 $\pm$ 10.7 (35)	<0.001
16.7	261.5 $\pm$ 11.9 (35)	275.4 $\pm$ 11.0 (35)	NS

Results expressed as mean $\pm$ SEM with number of observations in parentheses

Fig. 1. Effect of increasing concentrations of CGP 28392 (logarithmic scale) upon insulin release from islets incubated in the presence of D-glucose (7 mmol/l). Mean $\pm$ SEM values are shown with the number of individual measurements in parentheses. ----: the concentration of CGP 28392 causing a half-maximal increment in insulin output

Fig. 2. Effect of CGP 28392 (25 μ mol/l) administered from min 46 to 70 upon ^{45}Ca fractional outflow rate (FOR; upper panel) and insulin release (lower panel) from prelabeled pancreatic islets perfused in the presence of D-glucose (7.0 mmol/l). The experiments were performed either at normal Ca^{2+} concentration (1.0 mmol/l; closed circles) or in the absence of Ca^{2+} and presence of EGTA (0.5 mmol/l; open circles). Mean $\pm$ SEM values refer to four individual experiments in each case

presence of D-glucose in the 5.6–11.1 mmol/l range, but failed to affect significantly insulin release at either a non-stimulatory concentration of glucose (2.8 mmol/l) or at a high concentration of the sugar (16.7 mmol/l) causing close-to-maximal stimulation of secretion. Thus, the effect of CGP 28392 upon glucose-stimulated insulin release resulted in a shift to the left of the sigmoidal curve relating insulin output to the ambient glucose concentration.

At a fixed concentration of D-glucose (7.0 mmol/l), the effect of CGP 28392 upon insulin release was dose-related in the 1–100 μ mol/l range, with a half-maximal response at a drug concentration close to 7 μ mol/l (Fig. 1). However, when the concentration of CGP 28392 was raised to 0.5 mmol/l, the release of insulin evoked by D-glucose (7.0 mmol/l) was decreased to $55.9 \pm 12.2\%$ ($n=22$) of the corresponding mean control value. At a glucose concentration of 7.0 mmol/l, the secretory response to CGP 28392 (25 μ mol/l) was a rapid, biphasic and sustained phenomenon (Fig. 2). The stimulant action of CGP 28392 upon insulin output was already evident after 1 min and reached its peak value during min 3 of exposure to the drug. A different picture was seen when the same experiments were repeated at a lower concentration of glucose (2.8 mmol/l) and a somewhat higher concentration of CGP 28392 (50 μ mol/l). In such a case, the drug only caused a modest increase in insulin output, which reached its peak value after 9 min exposure to CGP 28392. After correction for basal output (25 ± 12 nU/min per islet), the drug-induced increment in insulin output reached, at min 54 of perfusion, a peak value of 28 ± 13 nU/min per islet and over 25 min stimulation averaged no more than 19 ± 4 nU/min per islet ($n=4$ in all cases).

The data summarized in Table 2 indicate that verapamil (0.01 mmol/l) inhibited to a greater relative extent the secretory response to D-glucose (7.0 mmol/l) than the increment in secretion attributable to CGP 28392 (50 μ mol/l). Indeed, relative to the appropriate control value, the inhibitory action of verapamil amounted to $75.4 \pm 4.6\%$ in response to D-glucose and $42.9 \pm 14.6\%$ in response to CGP 28392. CGP 28392 (50 μ mol/l) failed to affect insulin release evoked, in the absence of Ca^{2+} , by the association of Ba^{2+} and theophylline (Table 2).

Ionic data

In the presence of glucose (7.0 mmol/l), CGP 28392 in the 5–100 μ mol/l range, stimulated ^{45}Ca net uptake by the islets (Table 3). At the lowest efficient concentration (5 μ mol/l), CGP 28392 increased ^{45}Ca net uptake by $14.2 \pm 5.5\%$ above the mean control value ($p < 0.05$). At a 20-fold higher concentration (100 μ mol/l), the relative increase in ^{45}Ca net uptake amounted to $35.1 \pm 7.5\%$. In the presence of D-glucose (7.0 mmol/l), a still higher concentration of CGP 28392 (0.5 mmol/l) failed to enhance and actually inhibited ^{45}Ca net uptake.

Table 2. Effect of CGP 28392 upon insulin release in the presence of different insulintropic agents

D-glucose (mmol/l)	Ca ²⁺ (mmol/l)	Ba ²⁺ (mmol/l)	Theophylline (mmol/l)	Verapamil (mmol/l)	CGP 28392 (mmol/l)	Insulin output (μU/90 min per islet)
2.8	1.0	–	–	–	–	12.8 ± 1.5 (24)
7.0	1.0	–	–	–	–	49.5 ± 3.3 (24)
7.0	1.0	–	–	–	0.05	76.1 ± 4.7 (24)
7.0	1.0	–	–	0.01	–	21.6 ± 2.6 (24)
7.0	1.0	–	–	0.01	0.05	36.8 ± 2.8 (24)
–	–	2.0	1.4	–	–	127.0 ± 3.4 (29)
–	–	2.0	1.4	–	0.05	128.1 ± 4.2 (29)

Results expressed as mean ± SEM with number of observations in parentheses

Table 3. Effect of CGP 28392 upon ⁴⁵Ca net uptake by islets incubated in the presence of D-glucose (7.0 mmol/l)

CGP 28392 (μmol/l)	⁴⁵ Ca net uptake (pmol/islet at 90th min)
Nil	2.53 ± 0.08 (47)
1	2.75 ± 0.13 (15)
5	2.89 ± 0.14 (15)
10	2.99 ± 0.16 (17)
50	3.05 ± 0.15 (17)
100	3.50 ± 0.19 (15)
500	1.82 ± 0.09 (15)

Results expressed as mean ± SEM with number of observations in parentheses

At a fixed concentration of D-glucose (7 mmol/l) and increasing concentrations of CGP 28392 (1.0–500 μmol/l), there was a significant correlation between the mean values for ⁴⁵Ca uptake and insulin release ($r=0.932$, $n=7$, $p<0.01$). The relationship between these two variables [9] suggested that, in excess of a threshold value for ⁴⁵Ca uptake close to 1.5 pmol/islet (at min 90), the increment in insulin output relative to that in ⁴⁵Ca net uptake averaged 46 μU/pmol.

When the islets were prelabelled with ⁴⁵Ca and placed in a perfusion chamber, CGP 28392 (25 μmol/l) provoked, in the presence of glucose (7 mmol/l), a rapid increase in effluent radioactivity (Fig. 2). The latter increase was suppressed when the islets were perfused in the absence of extracellular Ca²⁺ and presence of EGTA (0.5 mmol/l), suggesting that it corresponded to a process of exchange between influent ⁴⁰Ca and effluent ⁴⁵Ca. Before CGP 28392 administration, the fractional outflow rate of ⁴⁵Ca from the islets exposed to glucose (7.0 mmol/l) was lower in the absence than presence of extracellular Ca²⁺ ($p<0.005$; Fig. 2). When these experiments were repeated at a lower glucose concentrations (2.8 mmol/l), the increment in ⁴⁵Ca fractional outflow rate evoked by CGP 28392 (50 μmol/l) in the presence of Ca²⁺ (1.0 mmol/l) was less marked and less rapid. Thus, the peak value in ⁴⁵Ca fractional outflow rate was reached 9 min after introduction of CGP 28392 and represented a mean increment of $0.047 \pm 0.006\%/min$ (paired difference between min 54 and 45) as distinct from $0.342 \pm 0.019\%/min$ in the experiments illustrated in Figure 2.

Discussion

The present results indicate that CGP 28392 reproduces the stimulant action of D-glucose upon Ca²⁺ entry into and insulin release from pancreatic islets. Thus, the calcium-agonist caused a shift to the left of the sigmoidal curve relating insulin output to glucose concentration. This coincided with stimulation of ⁴⁵Ca net uptake, which was measured in washed islets. The stimulation of ⁴⁵Ca net uptake was apparently attributable to facilitated Ca influx into the islet cells. Thus, CGP 28392 enhanced ⁴⁵Ca efflux from prelabelled islets, provided that Ca²⁺ was present in the perfusate. The drug failed to affect ⁴⁵Ca outflow from islets perfused in the absence of extracellular Ca²⁺, suggesting that it did not exert any primary effect upon either the intracellular handling or efflux of Ca²⁺. It is also unlikely that CGP 28392 acted upon a late event in the secretory sequence, since it failed to affect insulin release evoked, in the absence of Ca²⁺, by the combination of Ba²⁺ and theophylline. The latter process is currently attributed to an intracellular redistribution of Ca²⁺ in the islet cells [12, 13].

The calcium-agonist CGP 28392 was still able to augment insulin release in the presence of verapamil, which was used at a concentration sufficiently high to cause a severe inhibition of glucose-stimulated insulin release. We have recently reported that, under suitable experimental conditions, organic calcium-agonists may even protect the pancreatic B cell against the inhibitory action of chemically related calcium-antagonists [14].

It should be underlined that CGP 28392 markedly augmented glucose-stimulated insulin release, but exerted little effect upon secretion at a non-stimulating glucose concentration. This behaviour is not attributable to the use of an insufficient concentration of CGP 28392. Indeed, at much higher concentrations (≥ 0.5 mmol/l), CGP 28392 inhibited glucose-stimulated insulin release. It is remarkable that a comparable situation was already observed with another calcium-agonist [14]. It is currently believed, especially from experiments performed at high concentrations of extracellular K⁺, that the depolarization of the plasma membrane and subsequent gating of voltage-sensitive Ca²⁺ channels is sufficient to provoke bioelectrical activity and in-

sulin release, even when the islet cells are deprived of exogenous nutrient [15–17]. Therefore, the modest effect of CGP 28392 upon insulin release at a low glucose concentration could be taken as an indication that the drug is not quite as efficient as high K^+ in causing the gating of Ca channels. It is also conceivable that, at higher glucose concentrations, the sugar facilitates, by some biophysical effect, e.g. by fluidification of the plasma membrane [18], the access of CGP 28392 to or its effect upon the regulatory system involved in the gating or closing of calcium channels. Moreover, since it was recently reported that the prevailing effect of CGP 28392 is a prolongation of the mean open time of Ca channels in cultured cardiac cells [19], it is quite understandable that the drug exerted little effect at either low or very higher glucose concentrations, when spiking bioelectrical activity is either absent or continuous in the absence of the drug [20].

In conclusion, the present findings afford further support to the view that the gating of calcium channels represents an efficient modality for stimulation, or at least facilitation, of insulin release. This view should not be overlooked when considering alternative models of stimulus-secretion coupling, such as the mobilization of intracellular Ca^{2+} by inositol 1,4,5-triphosphate [21]. When taken in conjunction with previous observations [14, 22], the present findings also raise the question whether, in the process of nutrient-stimulated insulin release, endogenous dihydropyridine derivatives generated in the B-cell as a result of changes in redox state [23] participate in the remodelling of cationic fluxes.

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