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STIMULUS–SECRETION COUPLING OF ARGININE-INDUCED INSULIN RELEASE

INSULINOTROPIC ACTION OF AGMATINE

A. SENER, P. LEBRUN, F. BLACHIER and W. J. MALAISSE*

Laboratories of Experimental Medicine and Pharmacology, Brussels Free University, B-1000 Brussels, Belgium

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Abstract—Agmatine causes a concentration-related (0.1–1.0 mM) stimulation of insulin secretion by rat pancreatic islets exposed to D-glucose (5.6–11.1 mM) but, like L-arginine, fails to affect insulin release either in the absence of glucose or at a high concentration (16.7 mM) of the hexose. Agmatine, which accumulates in islet cells, also stimulates ^{45}Ca net uptake by the islets. The secretory response to agmatine represents a delayed process. It is enhanced by theophylline and suppressed by antimycin A or the absence of extracellular Ca^{2+}. The insulinotropic action of agmatine is compatible with the view that endogeneously formed polyamines may play a role in the secretory response of islet cells to L-arginine.

Recent reports have emphasized the possible role of polyamines in insulin release [1, 2]. It was also recently reported that exogenous L-arginine is converted to putrescine, spermidine and spermine in rat pancreatic islets [3]. The generation of these amines could thus conceivably play a role in the secretory response of the pancreatic B-cell to L-arginine. Indeed, 2-aminoisobutyrate, which increases ornithine decarboxylase activity in pancreatic islets enhances the release of insulin evoked by L-arginine, whereas D,L-α-difluoromethyl ornithine, which is a potent inhibitor of ornithine decarboxylase, exerts an opposite effect. In the present study, we have examined the effect of an exogenous polyamine, agmatine, upon insulin secretion by rat islets. Agmatine was selected for this study because it can be generated from L-arginine in bacteria but not in mammalian cells [4].

MATERIALS AND METHODS

Agmatine was purchased from Janssen Chimica (Beerse, Belgium). All experiments were performed with islets isolated from the pancreas of fed albino rats. The methods used to measure insulin release from incubated [5] or perifused [6] islets, the net uptake of ^{45}Ca [7] and its outflow from prelabelled islets [8] and the L-[1-^{14}C]glucose space of distribution [9] were identical to those described in the cited references. For measuring agmatine uptake, the islets were separated after incubation from the medium through a layer of oil [9]. A perchloric extract was then prepared from the islet pellet and its content in agmatine measured by a fluorescent technique after separation by high performance liquid chromatography, in the same manner as that described for the measurement of spermidine [10, 11]. The data were corrected both for the recovery of an agmatine standard and for extracellular contamination as judged from the L-glucose distribution space (2.60 ± 0.25 nl/islet; N = 21). No agmatine was detected in control islets incubated in the absence of this amine (P > 0.3; N = 5).

All results are expressed as the mean (±SEM) together with the number of individual observations. The statistical significance of differences was assessed by use of Student's *t*-test.

* To whom correspondence should be addressed at: Laboratoire de Médecine Expérimentale, 115, Boulevard de Waterloo, B-1000 Brussels, Belgium.

RESULTS

Agmatine uptake

After 10 min incubation in the presence of agmatine (1.0 mM), the islet content in this amine amounted, after correction for extracellular contamination, to 5.2 ± 1.3 pmol/islet (N = 4). No further increase in agmatine net uptake was recorded after 60 min incubation, in which case the readings averaged 6.4 ± 1.6 pmol/islet (N = 10).

Secretory response to agmatine

Agmatine (1.0 mM) did not affect insulin release either in the absence of D-glucose or at a very high concentration of the hexose (16.7 mM), but augmented significantly (P < 0.01 or less) insulin output at intermediate concentrations of D-glucose in the 5.6–11.1 mM range (Table 1). L-arginine, when tested at the same concentration (1.0 mM) as agmatine, failed to affect insulin secretion, whatever the concentration of D-glucose present in the medium. However, when the concentration of L-arginine was raised to 10.0 mM, the amino acid augmented insulin output at intermediate concentrations of D-glucose (5.6–11.1 mM), whilst still failing to affect insulin output either in the absence of the hexose or at the highest glucose level (16.7 mM). In other words, both agmatine (1.0 mM) and L-arginine (10.0 mM) caused a shift to the left of the sigmoidal relationship

Table 1. Effect of agmatine and L-arginine upon glucose-induced insulin release

D-Glucose (mM)	Control	Agmatine (1.0 mM)	L-Arginine (1.0 mM)	L-Arginine (10.0 mM)
Nil	27.7 ± 4.9 (12)*	23.8 ± 4.8 (13)	N.D.	34.2 ± 5.4 (13)
5.6	25.8 ± 1.7 (57)	53.6 ± 3.3 (64)	21.7 ± 3.7 (9)	54.8 ± 2.9 (19)
8.3	148.6 ± 14.1 (9)	244.9 ± 13.9 (9)	125.7 ± 14.2 (9)	225.6 ± 10.6 (9)
11.1	297.3 ± 15.1 (9)	358.7 ± 12.1 (9)	283.6 ± 13.6 (9)	344.4 ± 14.2 (9)
16.7	387.6 ± 13.4 (18)	391.5 ± 13.6 (16)	379.1 ± 19.0 (9)	415.0 ± 20.1 (9)

* All results are expressed as μU/islet per 90 min.
N.D. Not determined.

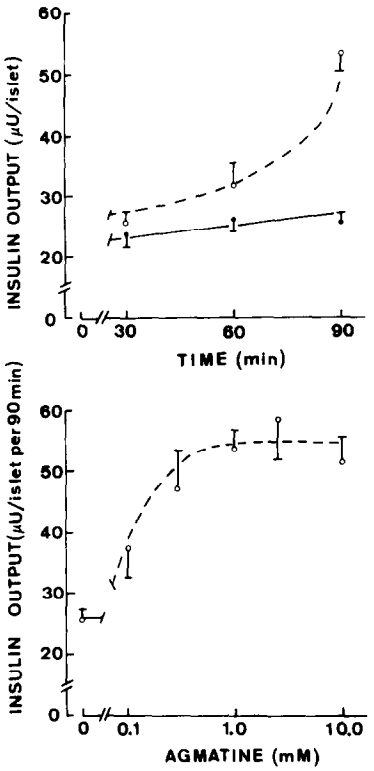

Fig. 1. Upper panel: time course for insulin release from islets incubated at 5.6 mM D-glucose in the absence (closed circles and solid line) or presence (open circles and dashed line) of agmatine (1.0 mM). Lower panel: effect of increasing concentrations of agmatine (logarithmic scale) upon insulin release from islets incubated for 90 min in the presence of 5.6 mM D-glucose. Mean values (±SEM) refer to 9–64 individual observations.

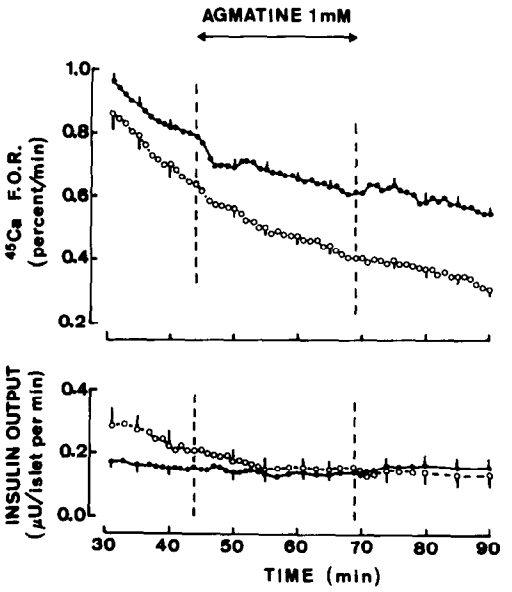

Fig. 2. Effect of agmatine (1.0 mM) administration from the 45th to 69th min upon ^{45}Ca fractional outflow rate (F.O.R.) and insulin output from islets perifused in the absence (closed circles) or presence (open circles) of D-glucose (5.6 mM). Mean values (±SEM) refer to 3–4 experiments for ^{45}Ca outflow and 7 experiments for insulin release.

between insulin output and D-glucose concentrations.

In the presence of 5.6 mM D-glucose, the insulinotropic action of agmatine was concentration-related in the 0.1–1.0 mM range, no significant further increase in insulin output being observed at higher concentrations of the polyamine (Fig. 1, lower panel). The time course for the secretory response to agmatine is illustrated in the upper panel of Fig. 1. No significant increase in insulin output could be detected over the first 30 min of exposure to agmatine, an obvious stimulation of insulin secretion being only recorded between the 60th and 90th min of incubation. The failure of agmatine to cause any immediate stimulation of insulin release was confirmed in both perifused islets (Fig. 2) and the isolated perfused rat pancreas (data not shown). It contrasts with the immediate increase in insulin output evoked by L-arginine in the same experimental models [12, 13].

The release of insulin evoked, in the presence of D-glucose (5.6 mM), by agmatine was considerably increased by theophylline (1.4 mM) and inhibited by either the absence of $CaCl_2$ or presence of antimycin A (Table 2). The response to theophylline was 4 times higher than that recorded in the sole presence of 5.6 mM D-glucose [14]. Agmatine (1.0 mM) also augmented insulin release evoked, in the absence of D-glucose, by L-leucine (10.0 mM) from a control value of 33.5 ± 3.5 to 54.5 ± 1.8 μU/islet per 90 min ($N = 9–10$; $P < 0.001$).

Table 2. Effect of Ca^{2+}, theophylline and antimycin A upon agmatine-stimulated insulin release

D-Glucose (mM)	Agmatine (mM)	Ca^{2+} (mM)	Theophylline (mM)	Antimycin A (mM)	Insulin output (μU/islet per 90 min)
5.6	1.0	1.0	—	—	50.3 ± 7.2 (18)
5.6	1.0	1.0	1.4	—	175.5 ± 6.6 (9)
5.6	1.0	—	—	—	21.3 ± 5.7 (7)
5.6	1.0	1.0	—	0.01	11.7 ± 4.1 (8)

Table 3. Effect of agmatine and L-arginine upon ^{45}Ca net uptake

D-Glucose (mM)	Agmatine (mM)	L-Arginine (mM)	^{45}Ca net uptake (pmol/islet at 90th min)
5.6	—	—	1.59 ± 0.12 (24)
5.6	1.0	—	2.22 ± 0.12 (24)
5.6	—	10.0	3.24 ± 0.17 (24)
5.6	1.0	10.0	7.99 ± 0.98 (24)

Cationic response to agmatine

The administration of agmatine (1.0 mM) to perifused islets failed to cause any obvious change in ^{45}Ca outflow (Fig. 2), whether in the absence or presence of D-glucose (5.6 mM). However, over 90 min incubation in the presence of 5.6 mM D-glucose, agmatine significantly increased ($P < 0.001$) the net uptake of ^{45}Ca by the islets (Table 3). Agmatine (1.0 mM) stimulated ^{45}Ca net uptake less efficiently ($P < 0.001$) than L-arginine (10.0 mM).

Combined effects of agmatine and L-arginine

After 90 min incubation at 5.6 mM D-glucose in the presence of both agmatine (1.0 mM) and L-arginine (10.0 mM), the net uptake of ^{45}Ca exceeded ($P < 0.001$) the value found, at the same hexose concentration and within the same experiments, in the sole presence of either agmatine or L-arginine (Table 3). However, the insulin output over 90 min incubation at 5.6 mM D-glucose in the presence of both agmatine and L-arginine amounted to 56.2 ± 2.5 μU/islet per 90 min ($N = 9$) and, hence, was virtually identical to that found, at the same hexose concentration and within the same experiments, in the sole presence of L-arginine (i.e. 54.8 ± 2.9 μU/islet per 90 min; $N = 19$).

DISCUSSION

It was already reported 15 years ago, in abstract form, that agmatine augments glucose-induced insulin release by isolated rat islets [15]. The present data reveal that agmatine is taken up by islet cells. The glucose-dependency of the insulinotropic action of agmatine was comparable to that evoked by L-arginine. The secretory response to agmatine was increased by theophylline and suppressed in the absence of extracellular Ca^{2+}. It coincided over 90 min incubation, with stimulation of ^{45}Ca net uptake. The secretory response of the islets to agmatine and L-arginine were not additive. These findings, which suggest that the accumulation of polyamines in islet cells may cause insulin release, are apparently compatible with the view that the secretory response to L-arginine may be related, to some extent, to the *de novo* generation of putrescine, spermidine and spermine from the cationic amino acid.

Several differences in the functional response to agmatine and L-arginine, respectively, need, however, to be underlined. First, whereas L-arginine causes immediate changes in cationic fluxes and insulin release, agmatine did not exert such immediate effects. Thus, agmatine failed to affect ^{45}Ca outflow and insulin release from perifused islets. Incidentally, whether in the absence or presence of D-glucose (5.6 mM), agmatine also failed to reproduce the rapid and sustained increase in ^{86}Rb outflow evoked by L-arginine (data not shown).

Second, whereas the secretory data collected in the presence of both agmatine and L-arginine could suggest a common mode of action, the measurement of ^{45}Ca net uptake indicated that the polyamine and amino acid exerted synergistic effects upon the latter cationic variable.

Last, the delayed time course for the cationic and secretory response to agmatine, as distinct from L-arginine, could not be accounted for by a late build-up in the agmatine content of islet cells.

The present results call, therefore, for a nuanced conclusion. Whilst the finding that an exogenous polyamine stimulates insulin release would be compatible with a role for endogenously formed polyamines in the secretory response to L-arginine, it is also conceivable that agmatine and L-arginine affect functional events in islet cells by two distinct modalities.

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