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REVIEW

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Anoctamin 1, candidate anionic channel sensitive to cell volume in insulin-producing cells

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ABSTRACT • It is proposed that anoctamin 1 represents a candidate as anionic channel sensitive to cell volume in insulin-producing cells. This proposal is based on experimental results concerning the expression of messenger RNA for this protein and its presence documented by immunohistochemistry in pancreatic islets and the effect of tannic acid and another inhibitor of anoctamin 1 upon the volume of islet cells, insulin secretion, glucose metabolism in pancreatic islets, the bioelectrical activity of beta cells and the transport of chloride anions activated by calcium via channels present in patches of plasma membrane excised from the same cells.

The present review deals with experimental findings suggesting that anoctamin 1 may act as an anionic channel sensitive to volume in insulin-producing cells.

As illustrated in Figure 1, the hypothesis concerning the participation of anionic channels sensitive to cell volume in the process of glucose-stimulated insulin release postulates that, following the entry of glucose in beta cells via the glucose transporter 2 (GLUT2) and its phosphorylation to glucose-6-phosphate catalyzed by glucokinase, the catabolism of the hexose provokes the intracellular accumulation of its metabolites, such as lactate and bicarbonate, the latter anion being generated from carbonic dioxide produced by the oxidation of glucose at the intervention of mitochondrial carbonic anhydrase. Such an intracellular accumulation of metabolites provokes in turn cell swelling in order to restore the equilibrium of cytosolic and extracellular osmotic pressure, the influx of water into the beta cells occurring at the intervention of an aquaglyceroporin such as AQP7. The increase in cell volume is itself responsible for the activation of anionic channels sensitive to cell volume, the gating of these channels then allowing the exit of anions, such as lactate, bicarbonate, chloride and orthophosphate. This escape of anions eventually leads to the depolarization of the plasma membrane and, hence, to the gating of calcium channels sensitive to membrane potential, to the inflow of calcium ions into the beta cell, to an increase in cytosolic calcium concentration and to the activation by calcium of the effector microtubular-microfilamentous system implied in the intracellular translocation of insulin secretory granules and their access to exocytotic sites (1-3).

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It should be underlined that the above sequence of events by no mean denies the consensual view according to which the cytosolic accumulation adenosine triphosphate (ATP) observed in response to an increase of extracellular glucose concentration and the acceleration of its catabolism leads to depolarization of the plasma membrane by closing potassium channels sensitive to ATP (Figure 1). As a matter of fact, the latter process prevails in the range of extracellular glucose concentrations below the threshold value for stimulation of insulin secretion, whilst the gating of anionic channel sensitive to cell volume represents a second component of the coupling device between stimulation and secretion, this second component being operative at higher glucose concentrations, i.e. when the closing of potassium channels sensitive to ATP has already reached its quasi-maximal value (4).

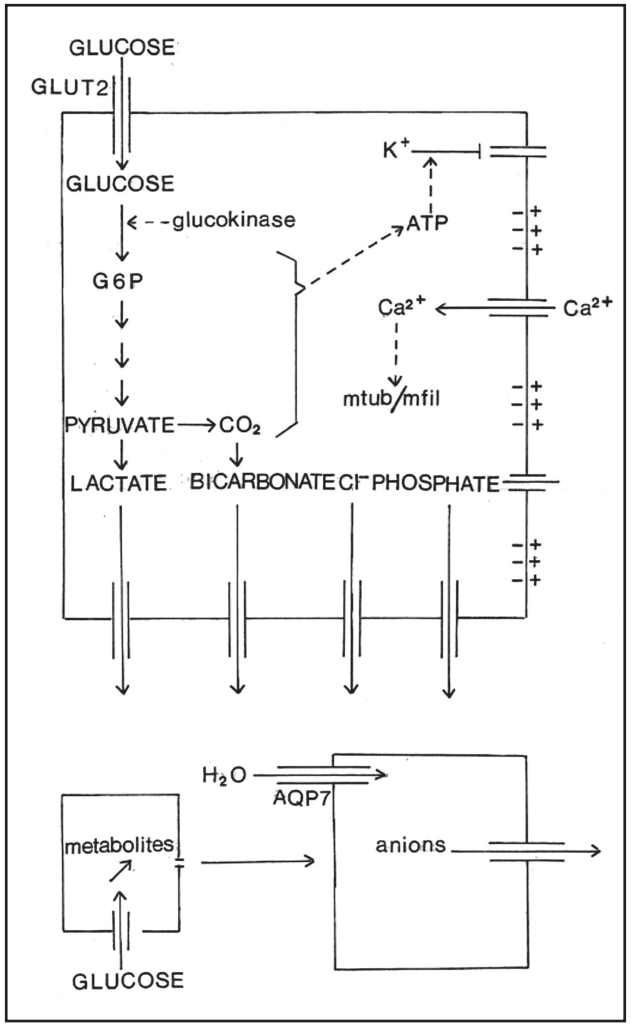

Figure 1 Schematic view of the stimulus-secretion coupling for glucose-stimulated insulin secretion.

The present hypothesis concerning the anionic channels sensitive to cell volume raises an obvious question, namely the identity of the major anionic channels implicated in such a process. The purpose of the present review is to propose that anoctamin 1 represents one, may be the principal, of these anionic channels (5, 6).

The presence of this protein in insulin-producing cells was documented by the expression of the RNA messenger for anoctamin 1 and by immunohistochemistry in both rodent and human islets (7).

A first functional experiment then allowed to assess the effect of tannic acid, an inhibitor of anoctamin 1, upon the volume of dispersed rat islet cells or tumoral BRIN-BD11 cells exposed to a hypotonic extracellular medium with an osmolarity decreased to one third of its normal value (Figure 2). The exposure of the cell to this hypotonic medium provoked within a few minutes an increase in cell volume rapidly followed by a later decrease of cell volume, the latter process being qualified as a regulatory volume decrease and being attributed to the efflux of anions *via* the anionic channels sensitive to cell volume. This process was suppressed in the presence of tannic acid (0.1 mM). Results comparable to those illustrated in Figure 2 were observed in the presence of an inhibitor considered more specific for anoctamin 1 than tannic acid, namely T16A inhibitor-AO1. Such was also the case in most other experiments mentioned in this review.

Figure 3 illustrates the effects of increasing concentrations of tannic acid upon the secretion of insulin provoked by glucose (16.7 mM) in incubated rat pancreatic islets, the broken line at the bottom of this figure indicating the basal value for insulin

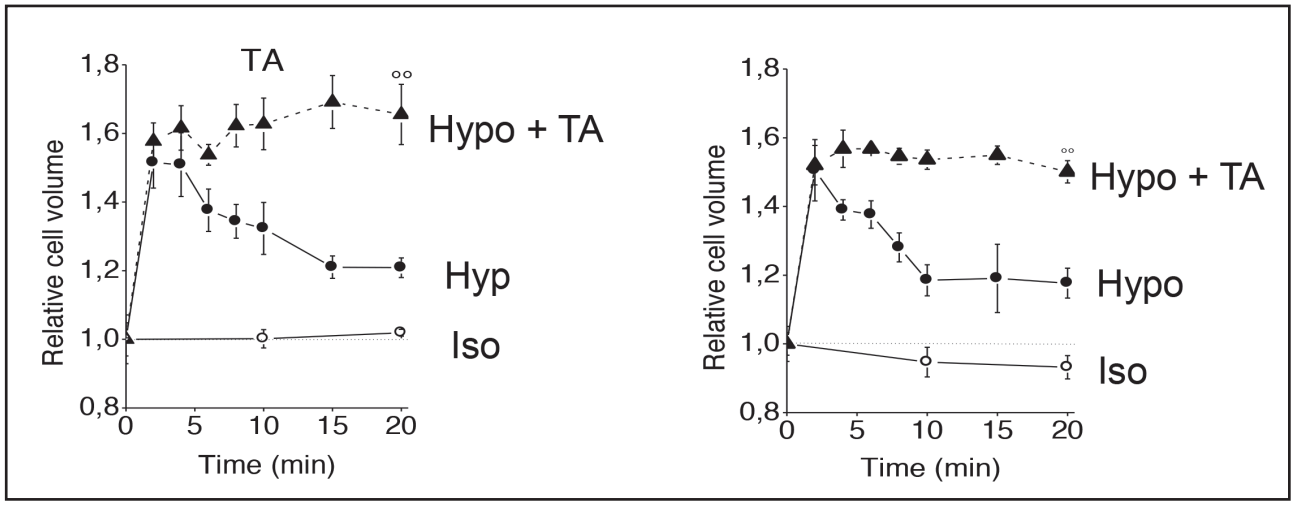

Figure 2 Volume of dispersed rat islet cells (left) and BRIN-BD11 cells (right) either maintained in an isotonic medium (open circles and solid line) or exposed to a hypotonic medium in the absence (closed circles and solid line) or presence (closed triangles and dashed line) of tannic acid (100 µM). Mean values (± SEM) are expressed relative to the initial volume of the cells. The hypotonic medium was obtained by a 50 mM reduction of its NaCl concentration. The °° indication refers to a probability below 0.01 for the effect of tannic acid on the cells exposed to the hypotonic medium.

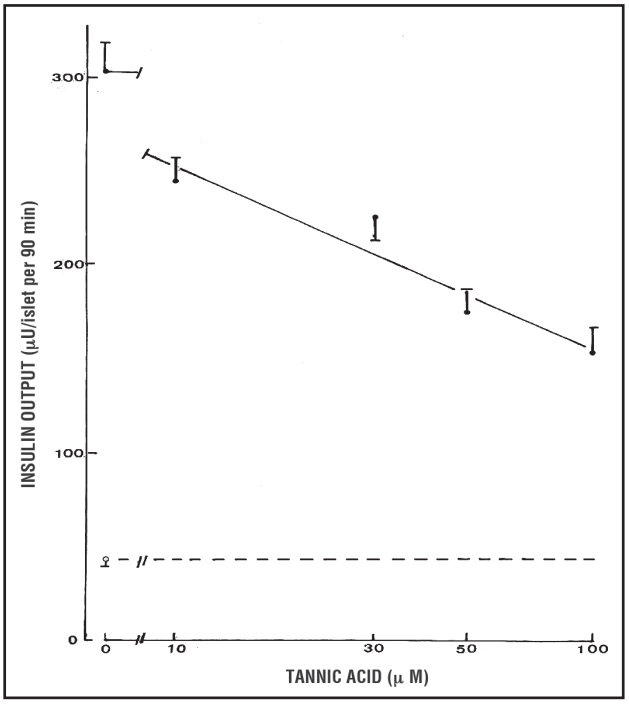

Figure 3 Effect of increasing concentrations of tannic acid (logarithmic scale) upon insulin release from rat islets incubated in the presence of 16.7 mM D-glucose (closed circles and solid line). The open circle refers to islets exposed to 2.8 mM D-glucose in the absence of tannic acid.

release measured in the presence of 2.8 mM glucose. The inhibitory effect of tannic acid upon glucose-stimulated insulin secretion is obvious. The secretory data listed in Table 1 confirm the inhibitory effect of tannic acid (100 µM) upon insulin release by islets exposed to 16.7 mM glucose. However, within the same experiments, tannic acid did not affect significantly insulin secretion recorded in the presence of only 8.3 mM glucose. These results suggest that the major role of anoctamin 1 concerns the insulin secretory response to high glucose concentrations.

Table 2 concerns the effect of tannic acid upon the metabolism of glucose in rat isolated islets. In the absence of tannic acid, a rise in glucose concentration from 2.8 to 16.7 mM augmented considerably the oxidation of glucose, as judged by the production of radioactive carbon dioxide from glucose uniformly labelled with carbon 14, as well as glucose utilization, as judged by the generation of tritiated water from glucose tritiated on carbon 5 of the hexose molecule. A preferential stimula-

Table 1 Effect of tannic acid upon glucose-stimulated insulin secretion from rat isolated islets

Expt.	D-glucose, mM	Tannic acid, μM	Insulin release, μU/islet per 90 min
1.	Nil	Nil	20.6±3.7 (19)
	8.3	Nil	78.7±7.2 (23)
	8.3	100	69.8±6.3 (24)
	16.7	Nil	357.2±34.8 (18)
	16.7	100	238.6±25.3 (20)
2.	8.3	Nil	79.0±6.7 (13)
	8.3	10	82.3±3.4 (15)
	8.3	30	81.2±3.5 (14)
	8.3	100	77.5±4.2 (11)

Table 2 Effect of tannic acid upon D-glucose metabolism. The results concerning $^{14}CO_2$ production from D-[U-^{14}C] glucose and ^{3}HOH production from D-[5-^{3}H]glucose are expressed as pmol of D-glucose equivalent/islet per 90 minutes. The paired ratio between D-[U-^{14}C]glucose oxidation and D-[5-^{3}H]glucose utilization is expressed as a percentage

D-glucose, mM	Tannic acid, μM	$^{14}CO_2$	^{3}HOH	$^{14}CO_2/^{3}HOH$, %
2.8	Nil	2.3±0.3 (26)	46.8±5.8 (27)	6.42±1.02 (25)
16.7	Nil	16.4±1.9 (27)	218.9±32.0 (25)	14.00±2.34 (23)
16.7	100	22.5±2.5 (28)	116.7±15.3 (21)	21.27±3.0 (21)

tion of the mitochondrial oxidative steps relative to glycolytic flux is documented by the increase in the ratio between $^{14}CO_2$ and ^{3}HOH production in response to the rise in glucose concentration from 2.8 to 16.7 mM. At the high concentration of glucose, tannic acid inhibited glucose utilization but, on the contrary, increased glucose oxidation. The determinants of these metabolic effects are likely to consist in an inhibition of glycolysis attributable to the intracellular accumulation of lactate and compensated by a stimulation of the mitochondrial oxidative steps of glucose catabolism. An essential finding emerging from these results consists in the fact that the total production of ATP generated by the catabolism of glucose was virtually identical in the absence or presence of tannic acid, averaging respectively 995 and 998 picomoles of ATP per islet over 90 minutes incubation. The inhibition of glucose-stimulated insulin secretion by tannic acid can thus not be attributed to any decrease in the energy yield of glucose catabolism in the islet cells.

Figure 4 illustrates the recording of membrane potential in mouse beta cells exposed either only to glucose (16.7 mM) or first stimulated by glucose in the absence and thereafter in the presence of tannic acid (100 μM). It is observed that tannic acid prolongs the duration of both the active and silent bioelectrical phases. As a matter of fact, Table 3 provides the mean values for different parameters of bioelectrical activity obtained in a series of seven similar experiments. The ascending branch of each peak of electrical activity corresponded to a depolarization of 39.1 millivolt in the absence of tannic acid, but only 26.2 millivolt in the presence of tannic acid. Tannic acid also decreased the number of spikes during the active phase from 4.9 to 3.3 peaks

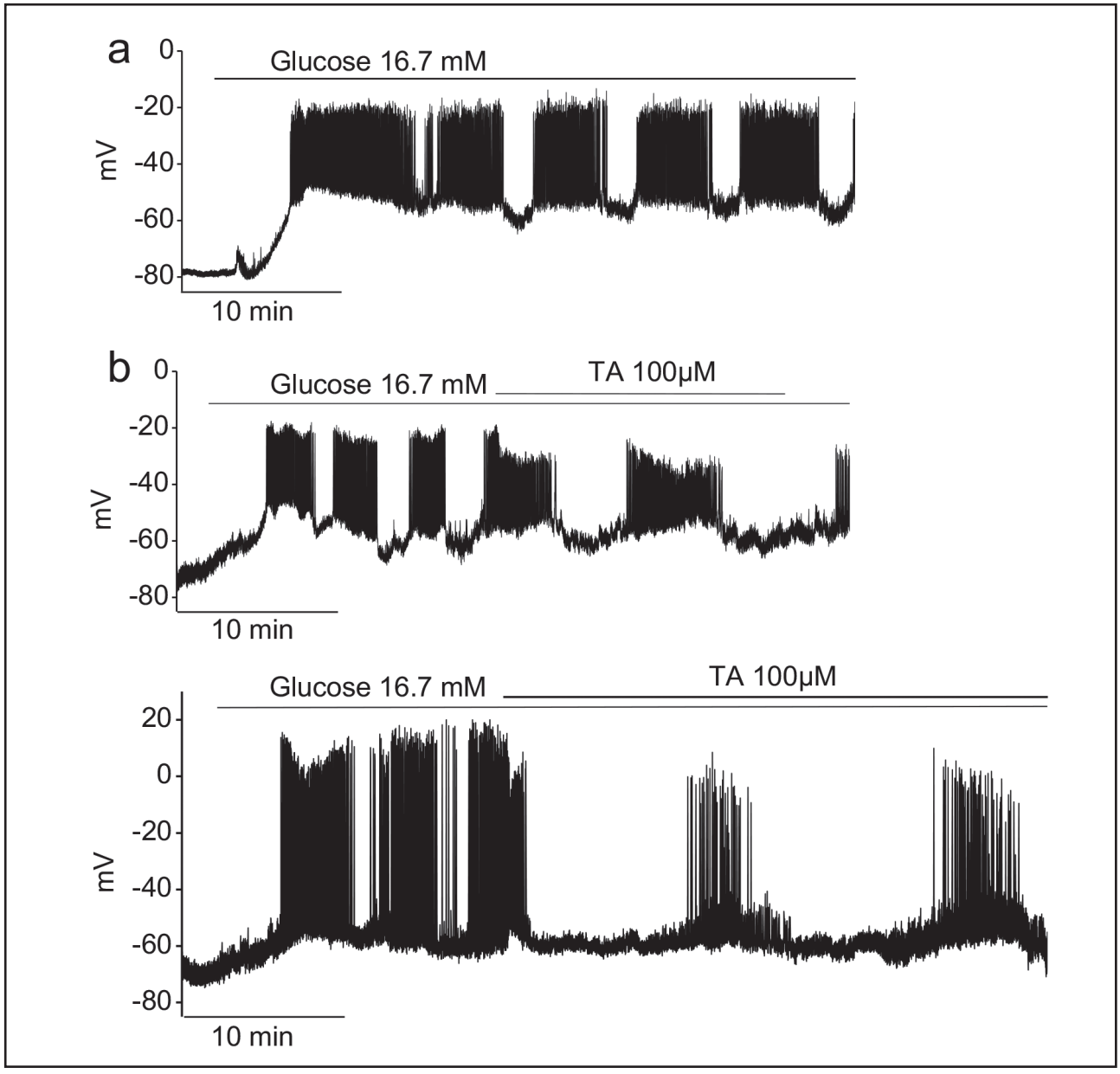

Figure 4 Effect of tannic acid (TA, 100 μM) on the membrane potential of mouse beta cells stimulated by D-glucose (16.7 mM).

per second. However, tannic acid augmented considerably the length of both the silent and active phases. Nevertheless, the relative duration of the active phases expressed per unit of time was somewhat decreased by tannic acid from about 65 to 59 percent. The number of peaks, over both the active and silent phases was thus decreased by tannic acid from about 3.2 to 1.9 peaks per second. When the latter data were multiplied by the mean height of action potential, it was observed that tannic acid decreased the sum of the active potentials from 124.1 to 51.1 millivolts per second, i.e. a reduction to about 41.2 percent of the control value. Since the ascending branch of each bioelectrical action peak

Table 3 Effects of tannic acid (TA) on parameters of beta cell electrical activity in mice islets

	TA (100 µM)		N
Resting potential (2.8 mM glucose)	-	- 75.7±0.1 mV	16
Burst characteristics (16.7 mM glucose)			
Silent phase			
Duration	-	98.8±14.2 s	7
	+	396.6±75.5 s	
Membrane potential	-	- 58.8±1.4 mV	7
	+	- 53.2±2.3 mV	
Active phase			
Duration	-	182.1±33.6 s	7
	+	573.1±103.1 s	
Membrane (plateau) potential	-	- 54.5±1.0 mV	9
	+	- 50.7±1.6 mV	
Top of action potential	-	- 15.4±3.7 mV	9
	+	- 24.5±3.0 mV	
Action potential height	-	39.1±4.4 mV	9
	+	26.2±3.9 mV	
Action potential number	-	4.91±0.62 spikes/s	7
	+	3.32±0.50 spikes/s	
Silent and active phases			
Action potential number	-	3.18 spikes/s	
	+	1.95 spikes/s	
Sum of action potential	-	124.1 mV/s	
	+	51.1 mV/s	

is currently attributed to the influx of calcium into the islet cells, such an analysis suggests that tannic acid decreases considerably the entry of calcium into insulin-producing cells. Such a decrease of calcium influx is likely to represent a major determinant of the inhibition of insulin release by tannic acid. In this respect, the mean tannic acid-induced decrease of estimated calcium influx to 41.2 percent of control value was virtually identical to the tannic acid-induced decrease of the secretory response to glucose above basal value, as illustrated in Figure 3, i.e. a reduction to 41.7 percent of control value.

Two last findings reinforce the idea that anoctamin 1 is indeed an essential candidate for the role of anion channel sensitive to cell volume in insulin-producing cells. First, the presence in the plasma membrane of rat beta cells of chloride channels activate by calcium was documented (Figure 5). The experimental design consisted in fragments of plasma membrane placed between a pipette and an incubation medium. The pipette contained a solution supposed to represent the extracellular medium and enriched with both the hypoglycemic sulphonylurea glibenclamide (10.0 µM) in order to insure the closing of potassium channels sensitive to ATP and the organic calcium antagonist nifedipine (also 10.0 µM) in order to close calcium channels sensitive to membrane potential. The incubation medium supposed to represent the cytosolic compartment was, when so required, enriched with ionized calcium (1.0 µM), i.e. a concentration close to the upper limit of physiological concentrations of ionized calcium in the cytosol of islet cells. In the presence of ionized calcium, the current intensity measured at potential differences from + 80 to - 60 millivolts indicated the opening of chloride channels with a conductance close to 8.4 picosiemens, similar to anoctamin 1 conductance reported in

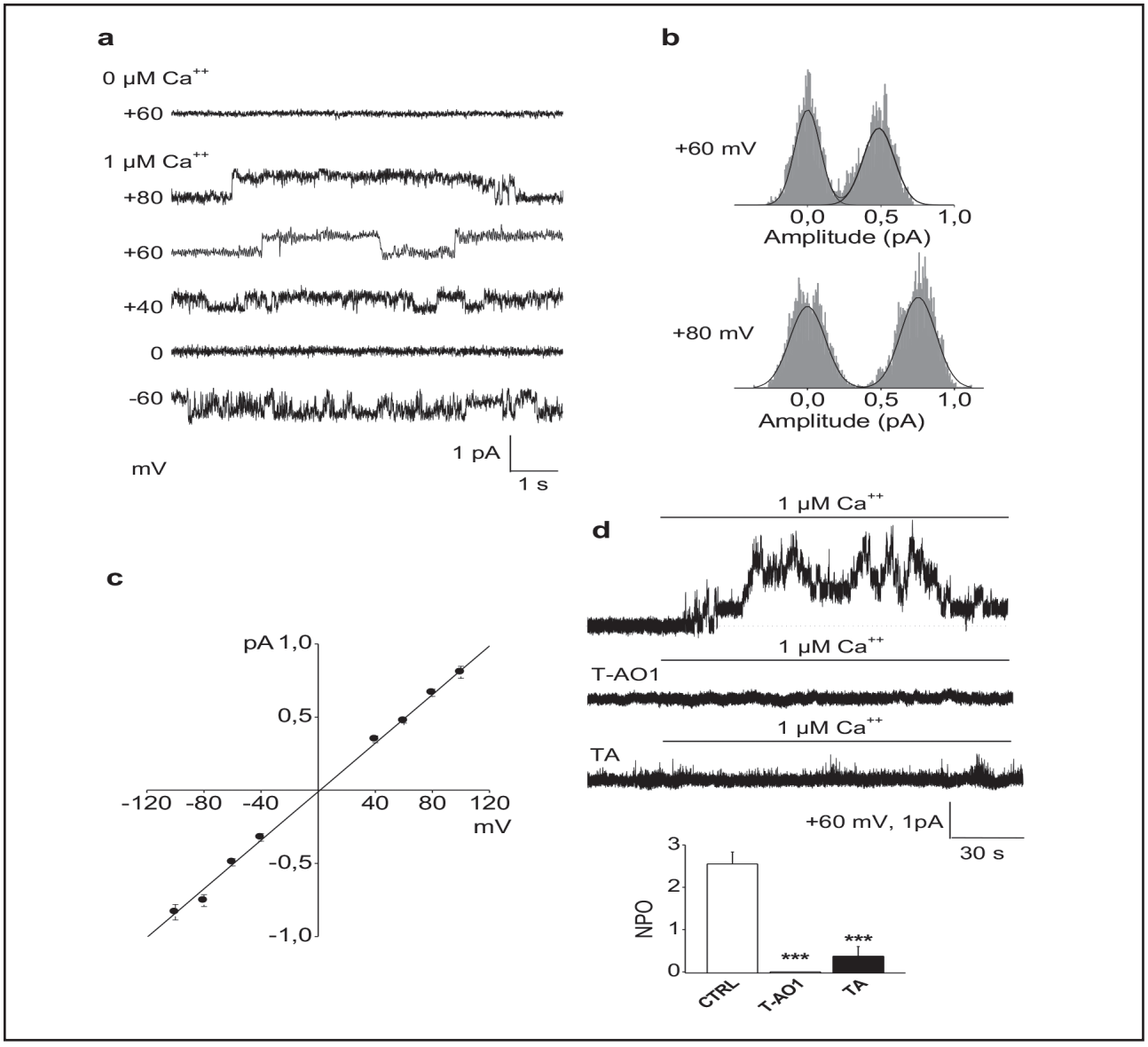

Figure 5 Single-channel Cl^- currents from inside-out patches excised from rat beta cells. Representative recordings of single channel currents activated by Ca^{2+} (1.0 μM) in the bathing solution corresponding to the intracellular compartment (panel a). Representative number of events - amplitude histograms at + 60 and + 80 mV, the scale bars corresponding to 250 events (b). Current-voltage relationship of single-channel Cl- currents activated by Ca^{2+}, a single-channel conductance of 8.4±0.2 pS being calculated from a linear fit (c). Time course of channel activity at + 60 mV before and during exposure to 1.0 μM Ca^{2+} in the absence of inhibitors, in the presence of T16A inhibitor-AO1 (100 μM) and in the presence of tannic acid (TA, 100 μM) in the pipette solution corresponding to the extracellular medium (d; upper records) and mean *NPo* values, i.e. the product of the number of channels in each patch (*N*) by the open probability (*Po*), calculated for 2 minutes after 15 seconds stimulation with Ca^{2+} (d; lower columns).

prior literature. In the plasma membrane of islet cells, tannic acid considerably decreased the mean opening probability (NPo) of these chloride channels activated by calcium. Taken as a whole, these findings document, in functional terms, the presence in the plasma membrane of insulin-producing cells an anionic channel the activation of which by cytosolic calcium allows the crossing of chloride anions (7).

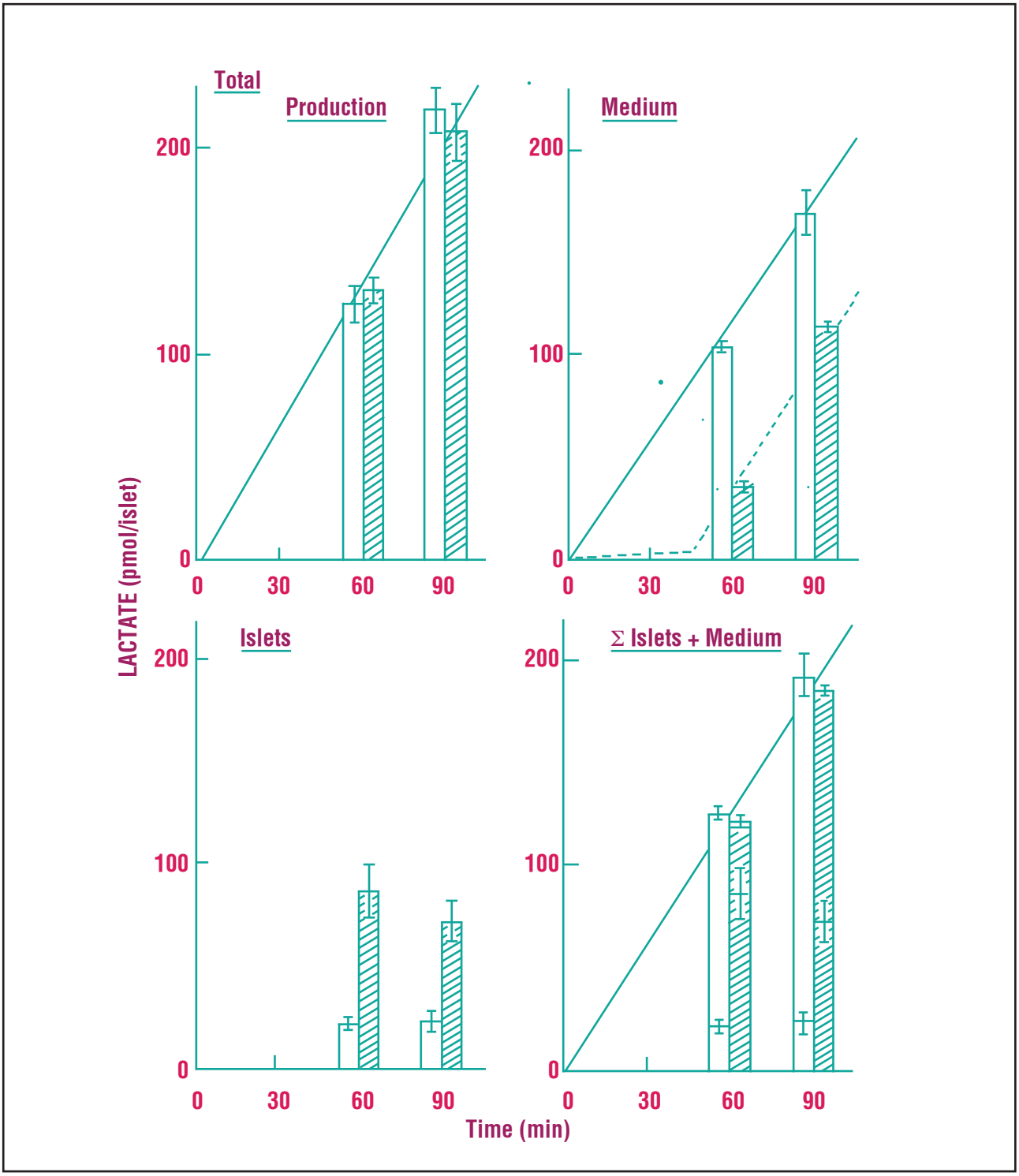

Figure 6 Isolated islets were incubated for 60 or 90 minutes with glucose (16.7 mM) in the absence (open columns) or presence (shaded columns) of verapamil (0.01 mM). After incubation, lactate was measured either after sonification of the islets in their incubation medium (total production; upper left panel) or separately in the medium (upper right panel) and in the islet homogenates (lower left panel), the sum of these two latter measurements being shown in the lower right panel). Each value represents the mean (± SEM) of four individual experiments.

Second, already in 1974, more than 40 years ago, we had initiated a series of investigations concerning the effects of organic calcium antagonists, such as verapamil, on different variables of islet function. Among others, it was observed that the total production of lactic acid by rat isolated pancreatic islets incubated during 60 or 90 minutes in the presence of glucose (16.7 mM) was not affected by verapamil (10.0 µM). However, the measurement of lactic acid in the incubation medium indicated that verapamil inhibits the outflow of lactic acid (Figure 6). This coincided with an increase of the lactic acid islet content during incubation in the presence of verapamil (8). It is only today that a reasonable interpretation may be offered to account for these unexpected experimental results. It may indeed be postulated that the inhibition by verapamil of calcium influx into the insulin-producing cells opposed the participation of anoctamin 1, an anionic channel susceptible to activation by calcium, to the efflux of lactate anions and, hence, provoked their intracellular accumulation.

In conclusion, anoctamin 1 seems indeed to represent an anionic channel sensitive to cell volume operative in insulin-producing cells.

REFERENCES

1. Malaisse WJ, Best L, Beauwens R, Sener A. Ionic determinants of the insulinotropic action of glucose: the anion channel hypothesis. Metab Funct Res Diab. 2005; 1:2-6
2. Malaisse WJ. Les déterminants ioniques de l'action insulinotrope des nutriments: non consensus omnium. Bull Mém Acad Roy Méd Belgique. 2008; 163:143-151
3. Best L, Brown PD, Sener A, Malaisse WJ. Electrical activity in pancreatic islet cells: the VRAC hypothesis. Islets. 2010; 2:59-64
4. Carpinelli AR, Malaisse WJ. Regulation of 86Rb outflow from pancreatic islets. V. The dual effect of nutrient secretagogues. J Physiol (London). 1981; 315:143-156
5. Malaisse WJ, Virreira M, Zhang Y, Crutzen R, Bulur N, Lybaert P, Golstein PE, Sener A, Beauwens R. Role of anoctamin 1 (TMEM16A) as a volume regulated anion channel in insulin-producing cells. Diabetologia. 2012; 55 (suppl. 1):S204
6. Malaisse WJ, Crutzen R, Bulur N, Virreira M, Rzajeva A, Golstein PE, Sener A, Beauwens R. Effects of the inhibitor of anoctamin 1, tannic acid, on insulin-producing cells. Diabetologia. 2013; 56 (suppl. 1):S196
7. Crutzen R, Virreira M, Markadieu N, Shlyonsky V, Sener A, Malaisse WJ, Beauwens R, Boom A, Golstein PE. Anoctamin 1 (Ano1) is required for glucose-induced membrane potential oscillations and insulin secretion by murine β-cells. Europ J Physiol. 2016; 468:573-591
8. Malaisse WJ, Herchuelz A, Levy J, Sener A. Calcium-antagonists and islet function. III. The possible site of action of verapamil. Biochem Pharmacol. 1977; 26:735-740