TETRAETHYLAMMONIUM POTENTIATION OF INSULIN RELEASE AND INHIBITION OF RUBIDIUM EFFLUX IN PANCREATIC ISLETS

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SUMMARY : The significance, for insulin release, of changes in B cell K permeability, was investigated with perifused rat islets. Tetraethylammonium (TEA, 20 mM) had no effect on insulin secretion at 2 mM glucose, but markedly and reversibly potentiated the stimulation by 6 mM glucose. TEA effect was not due to activation of cholinergic or β-adrenergic receptors in B cells. 6 mM glucose greatly reduced ^{86}Rb efflux (used as tracer of K efflux) from preloaded islets. At 2 mM glucose, TEA decreased ^{86}Rb efflux to the same extent as did 6 mM glucose alone; at 6 mM glucose TEA reduced ^{86}Rb efflux still further. These findings suggest that a decrease in K efflux from B cells is not sufficient to trigger insulin release, but may potentiate the secretory response evoked by a secretagogue.

Since the original work of Dean and Matthews (1), it has been well established that many insulin releasing agents produce depolarization and electrical activity in pancreatic islet cells and that a correlation exists between appearance of this activity and the stimulation of release (2-4). If the electrical properties of B cells have been well characterized (for a review, see 5), the ionic mechanisms underlying the changes in membrane potential are still largely unknown; changes in both active and passive ionic fluxes may be involved (6, 7). Using Rb as a substitute of K, Sehlin and Täljedal (8) suggested that the depolarizing effect of glucose on B cells might be mediated, at least in part, by a decrease in K permeability.

The purpose of the present study was to investigate whether a reduction in K permeability of B cells is sufficient to induce insulin release. Tetraethylammonium was used to this effect, since it is the most potent and specific

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blocker of K conductance in nerve and muscle cells (9-12); owing to its longer half-life, ^{86}Rb was used as tracer of K efflux from preloaded islets (8, 13).

MATERIALS AND METHODS

Isolated islets were obtained after collagenase digestion (14) of the pancreas of fed male Wistar rats (275-325 g) killed two hours after IP injection of 4 mg pilocarpine (15).

All experiments were performed at 37°C with Krebs-Ringer bicarbonate medium pH 7.4, supplemented with 0.5 % (w/v) bovine serum albumin and equilibrated with O_2/CO_2 (94:6). The perifusion system utilized to study insulin release has been previously described in detail (16); the same system was used to follow ^{86}Rb efflux from the islets, except for a smaller volume of the chamber (0.3 vs 0.8 ml) and a reduced flow rate (1.1 vs 2.6 ml/min). The dead time between the switch of the stopcock and collection of medium is 1 min and 45 sec for efflux and release setups respectively. In release experiments, groups of 15 islets were transferred into the chambers immediately after isolation and immunoreactive insulin (IRI) measured in the effluent fractions by a double antibody method (17), with rat insulin as standard. In efflux experiments, groups of 60 islets were first loaded with Rb by incubation for 2 hrs at 37°C, in 1 ml medium containing 2.8 mM glucose and 0.2 mM $^{86}RbCl$ (480 mCI/mmol). They were washed 3 times at room temperature with 5 ml medium and placed in the chamber. ^{86}Rb in the effluent fractions, collected at 1 min intervals, and remaining in the islets at the end of the experiment was counted in a liquid scintillation spectrometer. For each collection interval, the fractional efflux of ^{86}Rb (i.e. the fraction of label contained in the tissue that has been lost per min) was calculated. In preliminary experiments (6,6'-^{3}H)-sucrose was also added to the loading solution to label the extracellular space (18). Within 10 min of perifusion, all the sucrose marker was washed out.

Tetraethylammonium chloride (TEA, British Drug House) addition to the medium was compensated for by an appropriate decrease in NaCl ; $^{86}RbCl$ and (6,6-^{3}H)-sucrose were from the Radiochemical Center, Amersham; anti-insulin serum, rat insulin and propranolol were gifts from Dr. P.H. Wright, Dr. J. Schlichtkrull and ICI Belgium, respectively. Atropine and all other reagents were from Merck A.G.

RESULTS AND DISCUSSION

The basal rate of IRI release recorded at low glucose (2 mM) was not modified by 20 mM TEA (Fig. 1). In the presence of 6 mM glucose, IRI secretion was steadily stimulated and addition of TEA resulted in a rapid, biphasic and promptly reversible enhancement of the rate of release. After an early peak occuring at 3 min, IRI secretion slightly declined and stabilized. Upon removal of the drug, the secretion rate first fell below control values before returning to a comparable level (Fig. 1); such a biphasic decrease in secretion is also observed when glucose is reduced from a high to a lower, but still stimulatory concentration (19).

In view of the rich innervation of the islets (20) and since TEA is known

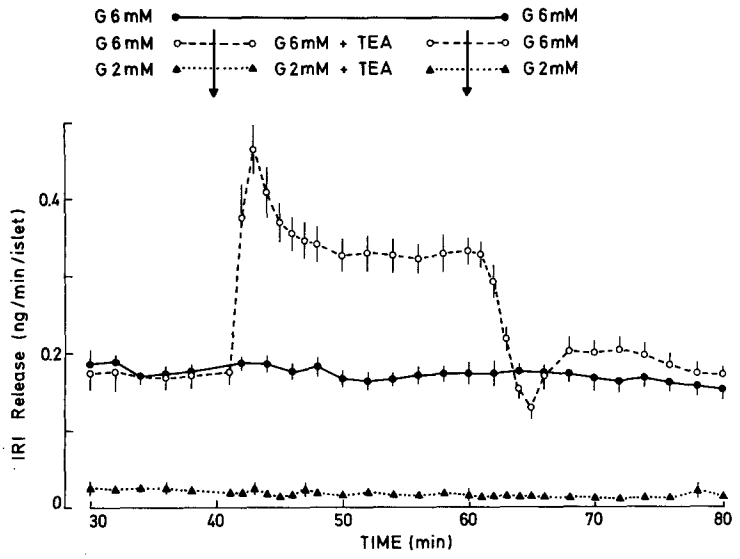

Fig. 1. Effect of 20 mM TEA on insulin (IRI) release by isolated rat islets perifused at glucose (G) 2 or 6 mM. The effluent was not collected during the first 30 min of perifusion. Values are means ± SEM of 4 experiments in G 6 mM alone and G 2 mM + TEA and of 8 experiments in G 6 mM + TEA.

to enhance neurotransmitter release in cholinergic (21) and adrenergic (22) nerves electrically stimulated, control experiments were performed to establish whether the potentiation of IRI release by TEA was mediated by activation of cholinergic (23) or β-adrenergic (24) receptors in B cells. Table 1 shows that the increment of secretion due to the drug was not significantly affected in the presence of the specific blockers of these receptors. In addition, the kinetics of the TEA effect was the same in the absence or the presence of atropine or propranolol (not shown). Another series of experiments was carried out to rule out that the decrease in the NaCl content of the medium accompanying TEA addition was responsible for the effects observed. Replacement of 20 mM NaCl by sucrose was without significant effect on IRI release induced by 6 mM glucose (Table 1).

^{86}Rb efflux from preloaded islets is shown in Fig.2. The rate of efflux was markedly smaller in the presence of 6 mM glucose than at 2 mM. This observation, made in a dynamic system and with a physiological glucose concentration,

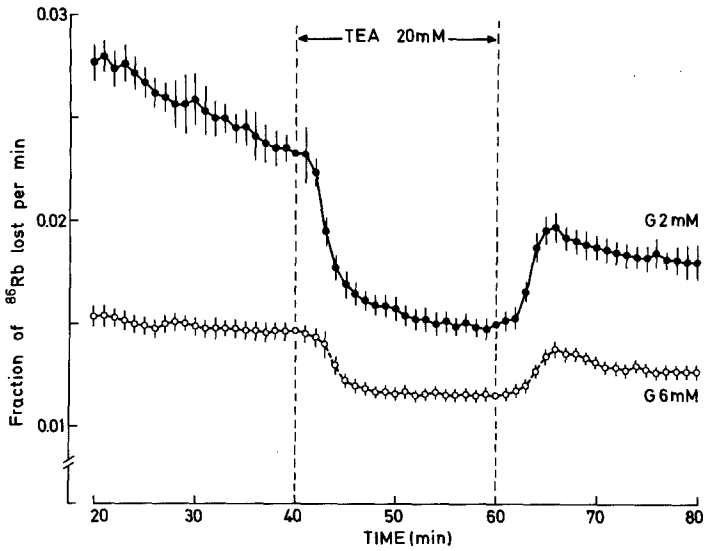

Fig. 2. Effect of 20 mM TEA on ^{86}Rb efflux from isolated rat islets perifused at glucose (G) 2 or 6 mM. Values are means ± SEM of 7 experiments at G 6 mM and of 5 experiments at G 2 mM.

Table 1. Effect of TEA on insulin release by perifused islets

Test condition	Increment of IRI release above controls (ng/islet/20 min)
TEA 20 mM	3.46 ± 0.26 (8)
TEA 20 mM + Atropine 10 μM	3.41 ± 0.12 (4)
TEA 20 mM + Propranolol 50 μM	3.26 ± 0.38 (4)
Sucrose 40 mM	0.01 ± 0.14 (4)

The experimental protocol was the same as that shown in Figure I. After 40 min of perifusion at glucose 6 mM, TEA or sucrose was added, for 20 min, and NaCl decreased by 20 mM. When present, atropine or propranolol was added to the medium from the start of the experiment. The increment in insulin secretion produced by the test condition was calculated as the total release between min 40 and 60, above a constant rate of secretion evaluated from the last 5 min of the initial equilibration period (min 35-40). Values are means ± SEM of a number of experiments given in parentheses.

is in agreement with the previous report (8) that islets incubated for 10 min in very high glucose (20 mM) retained more ^{86}Rb than islets incubated at 3 mM glucose. Addition of 20 mM TEA to the perifusate reversibly inhibited ^{86}Rb efflux from preloaded islets. The fall in ^{86}Rb efflux rate produced by TEA was

monophasic and reached a new stable level within 6 to 8 min. The changes in efflux were more important and slightly faster at 2 mM glucose; however, the slower rate of efflux recorded at 6 mM glucose was further reduced by TEA, what suggests a certain synergism between both agents.

In conclusion, TEA and glucose reduced Rb efflux from pancreatic islet cells, but the striking difference between the two agents is that TEA does not stimulate insulin release in absence of sufficient glucose, even when it decreases the efflux rate to the same extent as an effective glucose concentration. Combined with 6 mM glucose, TEA appears, however, to potentiate the glucose effect on both the inhibition of Rb efflux and stimulation of insulin release. Provided Rb is accepted as a valid tracer of passive K fluxes, these results suggest that a reduction in K permeability in B cells does not represent a sufficient signal for stimulating insulin release. The decrease in K conductance produced by a rise of glucose concentration just above the threshold for stimulation of release might explain the B cell depolarization from the resting membrane potential to the constant threshold potential, at which the sugar induces a regular electrical activity characterized by its burst pattern (5). A further reduction of K permeability in B cells by higher glucose concentrations (or by TEA) might then increase the burst duration (5) and the magnitude of the concomitant secretory response.

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