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CHOLINERGIC STIMULATION OF ION FLUXES IN PANCREATIC ISLETS

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Abstract—Cholinergic agents are known to stimulate the hydrolysis of polyphosphoinositides in pancreatic islets. The effect of carbamylcholine upon ion fluxes in the islet cells was investigated. Carbamylcholine provoked a rapid but poorly sustained increase in ^{45}Ca and ^{86}Rb outflow from perifused islets. Such a cationic response was observed at different glucose concentrations (zero to 16.7 mM), at three concentrations of carbamylcholine (10 μM, 100 μM and 1.0 mM), and in the absence or presence of extracellular Ca^{2+}. It coincided with a biphasic stimulation of insulin release, both the cationic and secretory responses being abolished in the presence of atropine (10 μM). At variance with nutrient secretagogues, carbamylcholine failed to affect the net production of cyclic AMP and caused a transient decrease in ^{32}P outflow from islets prelabelled with [^{32}P]phosphate. It is proposed that cholinergic agents mobilize Ca^{2+} from intracellular sites, possibly through generation of inositol, 1,4,5-triphosphate from phosphatidylinositol 4,5-bisphosphate. The intracellular redistribution of Ca^{2+} does not appear sufficient, however, to account fully for the secretory response, which may also involve activation of protein kinase C by diacylglycerol.

Among the several nutrient, hormonal and neural factors involved in the immediate and direct regulation of insulin release, cholinergic neurotransmitters are thought to participate in the stimulation of the pancreatic B-cell during the cephalic and later phases of insulin secretion associated with food intake [1]. It was recently reported that cholinergic agents are potent stimulators of the hydrolysis of phosphoinositides, especially phosphatidylinositol 4,5-bisphosphate, in pancreatic islets [2–4]. According to current views, the subsequent liberation of diacylglycerol and inositol 1,4,5-triphosphate may lead, respectively, to the activation of protein kinase C [5, 6] and mobilization of Ca^{2+} from non-mitochondrial intracellular organelles [7, 8]. In the light of these considerations, we have investigated in the present study the effect of carbamylcholine upon the ionic and secretory behaviour of perifused pancreatic rat islets.

MATERIALS AND METHODS

All experiments were performed with islets isolated [9] from the pancreas of fed albino rats. The methods used to measure insulin release [10] and the fractional outflow rate (FOR) of ^{32}P [11], ^{86}Rb [12] and ^{45}Ca [13] from perifused islets were described in detail elsewhere. Briefly, groups of 100 islets each were preincubated for 60 min in media (125 μl) containing D-glucose (16.7 mM), ^{45}Ca and/or ^{86}Rb, and then placed in a perifusion chamber. The flow rate amounted to 1.0 ml/min. In most experiments, insulin release, ^{45}Ca and ^{86}Rb outflow were measured simultaneously [14].

The production of cyclic AMP by the islets over a 30 min incubation was measured by radioimmunoassay, as described elsewhere [15].

Carbamylcholine chloride and atropine (free base) were purchased from Sigma Chemical Company (St. Louis, MO).

All results are presented as the mean (± S.E.M.) together with the number of individual experiments (N). In all figures, the vertical dotted lines correspond approximately to the time at which a new perifusate reached the collecting vials, allowance being made for the dead space of the perifusion device.

RESULTS

The effect of carbamylcholine (1.0 mM) upon ^{45}Ca FOR, ^{86}Rb FOR and insulin release from islets perifused at normal Ca^{2+} concentration (1.0 mM) is illustrated in Fig. 1. The experiments were performed in the presence of increasing concentrations of D-glucose (5.6, 8.3 and 16.7 mM). As expected from previous studies [16, 17], the ^{45}Ca FOR and insulin output, prior to carbamylcholine administration, both increased as a function of the glucose concentration. The ^{86}Rb FOR was lowest at the intermediate concentration of glucose (8.3 mM), also in good agreement with prior findings [18, 19]. In all cases carbamylcholine provoked a rapid increase in ^{45}Ca and ^{86}Rb FOR. Although such an increase represented mainly a peak-shaped phenomenon, the impression was gained that a modest stimulation of ^{45}Ca and ^{86}Rb outflow persisted throughout the period of exposure to carbamylcholine. Indeed, in

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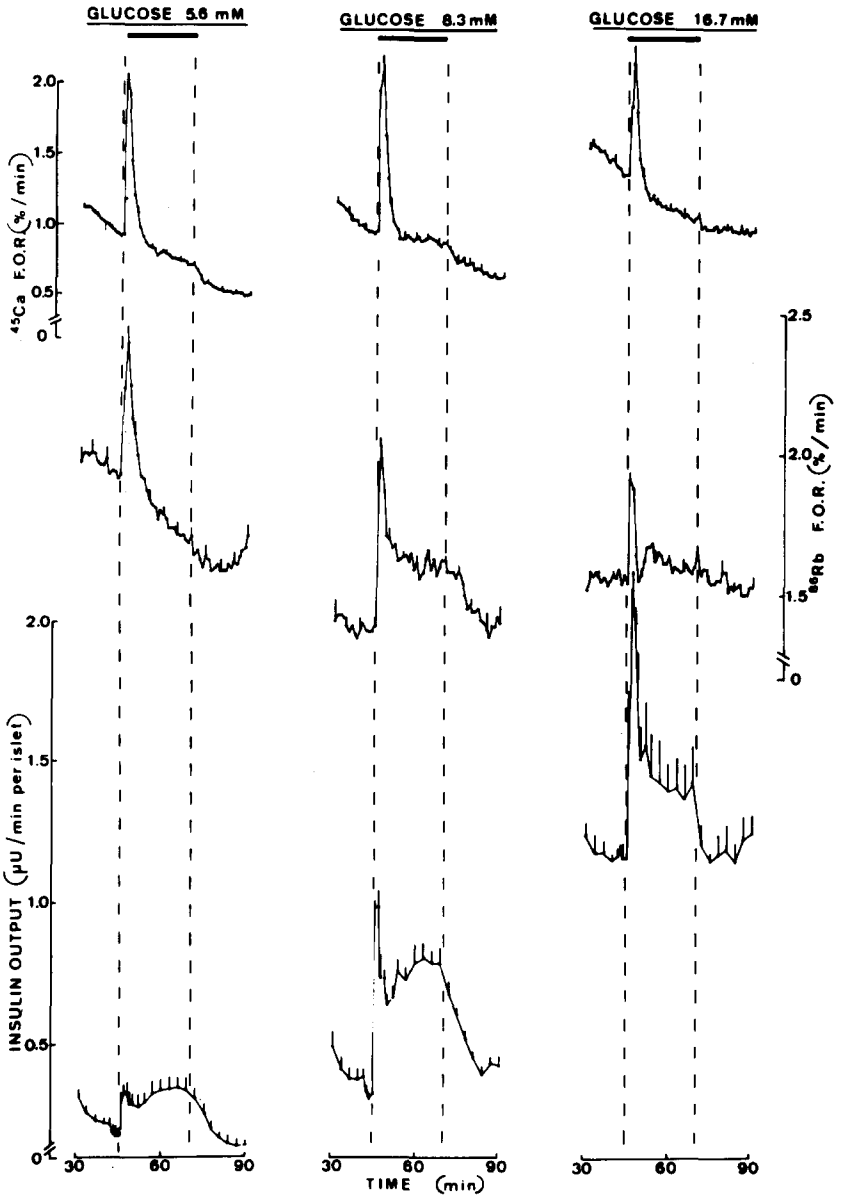

Fig. 1. Effect of carbamylcholine (1.0 mM), administered from min 46 to 69 (heavy bars at the top of the figure) upon ^{45}Ca and ^{86}Rb FOR and insulin release from islets perifused at normal Ca^{2+} concentration (1.0 mM) in the presence of 5.6 (left), 8.3 (middle) of 16.7 mM D-glucose (right). Mean values (± S.E.M.) refer, from left to right, to 13, 4 and 4 experiments.

most experiments, a slight fall in ^{45}Ca and ^{86}Rb FOR was seen after the 70th min of perifusion, when carbamylcholine was removed from the perifusate. At all glucose concentrations, carbamylcholine provoked a biphasic increase in insulin output.

Figure 2 illustrates the effect of increasing concentrations of carbamylcholine (10 μM, 100 μM and 1.0 mM) at a fixed concentration of D-glucose (5.6 mM). In all cases, a rapid increase in both ^{45}Ca and ^{86}Rb FOR was observed. The cationic response was lower at 10 μM carbamylcholine than at higher concentrations of the drug. In all cases, carbamylcholine also enhanced insulin release. The magnitude of such a stimulation was positively related to the concentration of carbamylcholine, especially during the late phase of the secretory response.

At the lowest concentration used in the present experiments (10 μM), carbamylcholine always stimulated ^{45}Ca FOR (Fig. 3), whether in the absence or presence of D-glucose (2.8–16.7 mM).

As illustrated in Fig. 4, atropine (10 μM), when administered throughout the perifusion period, virtually abolished the increase in ^{45}Ca FOR and insulin release normally evoked by carbamylcholine (1.0 mM) in the presence of 8.3 mM D-glucose. Incidentally, in these experiments, atropine failed to affect ^{45}Ca outflow and insulin release from the islets

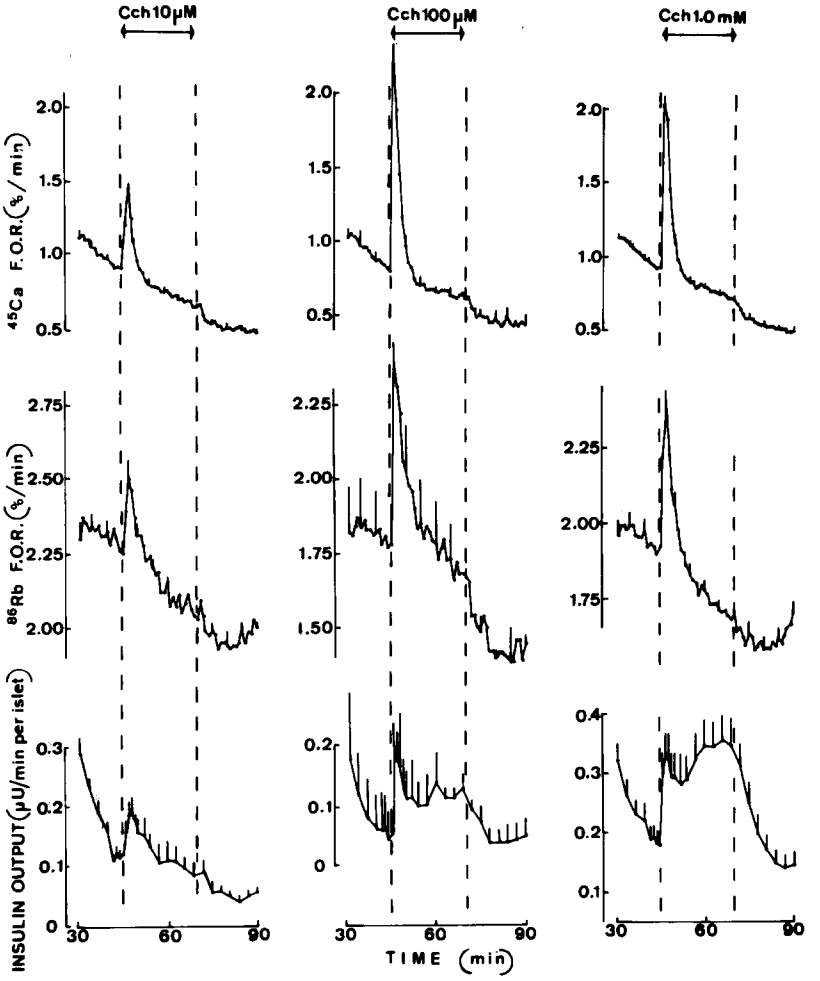

Fig. 2. Effect of increasing concentrations of carbamylcholine (10 μM: left; 100 μM: middle; 1.0 mM: right), administered from min 46 to 69, upon ^{45}Ca and ^{86}Rb FOR and insulin release from islets perifused at normal Ca^{2+} concentration (1.0 mM) in the presence of 5.6 mM D-glucose. Mean values (± S.E.M.) refer, from left to right, to 4, 2 and 13 experiments. Note that the ordinates axes were positioned so that the mean prestimulatory values (min 31 to 45) for each variable appear at the same level in the 3 series of experiments.

stimulated by D-glucose prior to the administration of carbamylcholine.

The increase in ^{45}Ca FOR evoked by carbamylcholine persisted in the absence of extracellular Ca^{2+}. In the presence of 8.3 mM D-glucose, the simultaneous removal of Ca^{2+} and introduction of EGTA provoked a transient increase in ^{45}Ca FOR (Fig. 5), in good agreement with a prior observation [20]. When carbamylcholine (1.0 mM) was introduced 10 min thereafter, the cholinergic agent provoked a rapid and transient increase in ^{45}Ca FOR. Even when the islets were perifused throughout in the absence of Ca^{2+} and presence of EGTA (0.5 mM) and D-glucose (4.4 mM), the administration of carbamylcholine (10 μM) still caused a rapid and transient increase in both ^{45}Ca and ^{86}Rb FOR (Fig. 6). The secretory response to carbamylcholine was suppressed, however, in the absence of Ca^{2+}. For instance, in the experiments illustrated in Fig. 5, the difference in mean secretory rate between carbamylcholine-stimulated and control islets averaged no more than 0.12 ± 0.11 μU/min per islet (degree of freedom = 15), as computed over 15 min exposure to the cholinergic agent.

In a further series of experiments, performed at normal Ca^{2+} concentration (1.0 mM), the effect of carbamylcholine (1.0 mM) upon ^{32}P FOR was examined in the presence of 8.3 mM D-glucose (Fig. 7). Whereas a rise in D-glucose concentration from 8.3 to 16.7 mM provoked a transient increase in ^{32}P FOR, no phosphate flush was seen in response to the administration of carbamylcholine. On the contrary, the cholinergic agent provoked a transient decrease in ^{32}P FOR.

Over 30 min incubation in the presence of 5.6 mM D-glucose and 1.0 mM Ca^{2+}, carbamylcholine (1.0 mM) failed to affect the production of cyclic AMP, which averaged 8.1 ± 0.9 and 7.3 ± 0.7 fmol/islet (N = 23 in each case) in the absence and presence of the cholinergic agent, respectively.

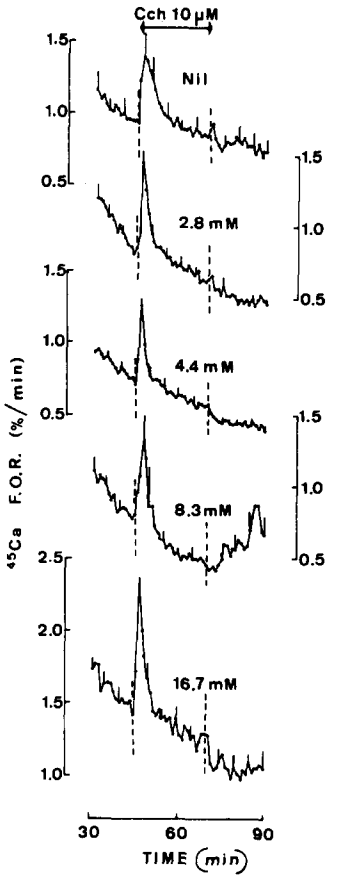

Fig. 3. Effect of carbamylcholine (10 μM), administered from min 46 to 69, upon ^{45}Ca FOR from islets perifused at normal Ca^{2+} concentration (1.0 mM) in the absence of glucose or at increasing concentrations of the hexose. Mean values (± S.E.M.) refer to 4 experiments in each case.

DISCUSSION

It is known from previous studies that cholinergic agents stimulate insulin release [21–24]. As a rule, this effect is negligible in the absence of glucose or at low glucose concentrations, most evident at intermediate concentrations of the hexose in the 5–10 mM range, but still present at high glucose levels (17–20 mM) [21, 22, 24, 25]. The secretory response to cholinergic agents coincides with enhanced bioelectrical activity [26] and is suppressed in the absence of extracellular Ca^{2+} or presence of verapamil [21, 24]. Cholinergic agents fail to exert any obvious effect upon glucose oxidation [26] and the cyclic AMP or cyclic GMP content of the islets [22, 24].

Relatively little information is available on the effects of cholinergic agents upon ionic movements in islet cells.

Gagerman *et al.* [26] reported that cholinergic agents increase $^{22}Na^{+}$ uptake, fail to affect either $^{36}Cl^{-}$ retention or $^{86}Rb^{+}$ uptake (at least in the presence of 11.1 mM glucose), and exert an effect opposite to that of glucose upon the fluorescence of chlorotetracycline-stained islet cells. In the same study performed with islets from hereditarily obese mice, acetylcholine, used in combination with eserine, did not stimulate ^{45}Ca uptake, except at a low glucose concentration (3 mM) in which case a modest increase in ^{45}Ca uptake failed to be associated with any stimulation of insulin release. Wollheim *et al.* [24] also observed a dissociation between the effects of cholinergic agents and glucose, respectively, upon ^{45}Ca uptake and insulin release. For instance, at a high glucose concentration (16.7 mM), acetylcholine enhanced insulin release whilst failing to affect ^{45}Ca uptake.

All these ionic data were collected in static incu-

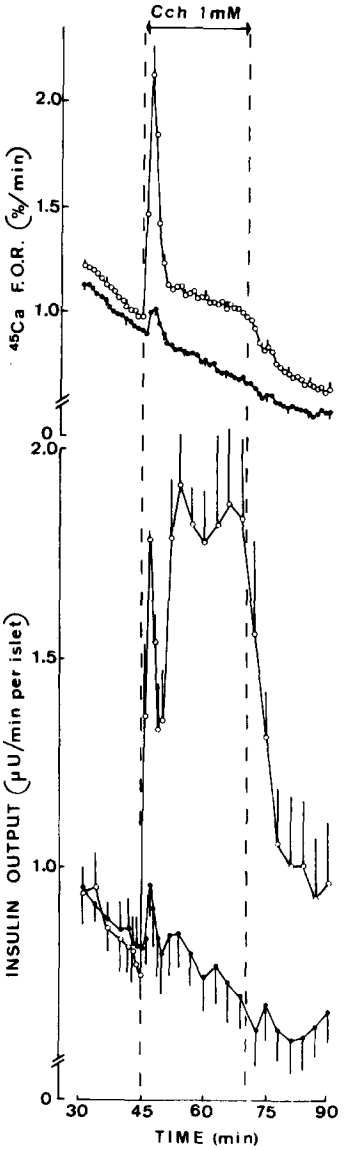

Fig. 4. Effect of carbamylcholine (1.0 mM), administered from min 46 to 69, upon ^{45}Ca FOR and insulin release from islets perifused at normal Ca^{2+} concentration (1.0 mM) in the presence of 8.3 mM D-glucose. In one series of experiments (closed circles), atropine (10 μM) was administered throughout the 90 min of perifusion. Mean values (± S.E.M.) refer to 4 experiments in each case.

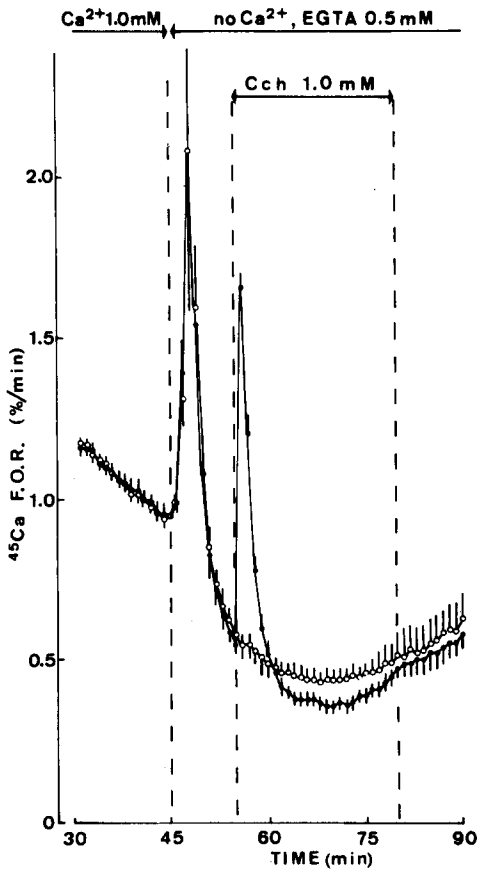

Fig. 5. Effect of extracellular Ca^{2+} upon the stimulation by carbamylcholine of ^{45}Ca FOR. The islets were perifused for 45 min at normal Ca^{2+} concentration (1.0 mM). From the 46th min onwards, the perifusate was deprived of $CaCl_2$ and enriched with EGTA (0.5 mM). In one series of experiments (●—●), carbamylcholine (1.0 mM) was administered from min 56 to 80. All media contained D-glucose (8.3 mM). Mean values (± S.E.M.) refer to 7 (control) and 10 (test) experiments.

bations and, when this work was undertaken, no information was available on the dynamic aspects of the ionic response to cholinergic agents. The present results, which are in good agreement with those reported in a recent publication [27], indicate that such a response is vastly different from that evoked by nutrient secretagogues. First, whereas the latter provoke a transient increase in ^{32}P [11, 28], a phenomenon known as the phosphate flush, carbamylcholine caused a transient decrease in ^{32}P FOR. Incidentally, our data confirm that the phosphate flush is not the reflection of phosphoinositides breakdown. Second, whereas nutrient secretagogues usually decrease ^{86}Rb FOR [12, 18], carbamylcholine caused a rapid increase in ^{86}Rb outflow. Last, whereas the stimulation of ^{45}Ca FOR by nutrient secretagogues reflects stimulation of $^{40}Ca^{2+}$ influx and, as such, is suppressed in the absence of extracellular Ca^{2+} [10, 16], the stimulation of ^{45}Ca FOR by carbamylcholine persisted in the absence of extracellular Ca^{2+}.

The mechanisms responsible for the carbamylcholine-induced changes in ^{32}P and ^{86}Rb FOR are open to speculation. For instance, the increase in ^{86}Rb outflow could reflect stimulation of a Ca^{2+}-dependent modality of K^+ extrusion [18, 19]. However, since the increase in ^{86}Rb FOR persisted in the absence of extracellular Ca^{2+}, which abolished the secretory response to carbamylcholine, it could also correspond to the gating of voltage-sensitive K^+ channels or an intracellular redistribution of $^{86}Rb^+$ [29, 30].

The carbamylcholine-induced increase in ^{45}Ca FOR apparently corresponds to a muscarinic process, blocked by atropine. It was observed at all glucose concentrations. It may reflect mobilization of ^{45}Ca from cellular sites, being still observed in the absence of extracellular Ca^{2+}. It is conceivable that such an intracellular redistribution of ^{45}Ca is attributable to the release of inositol 1,4,5-triphosphate from phosphatidylinositol 4,5-bisphosphate and participates in the secretory response to cholinergic agents. However, the carbamylcholine-induced increase in ^{3}H outflow from islets prelabelled with myo-[2-^{3}H(N)]inositol is decreased, but not abolished, in the absence of extracellular Ca^{2+} [31], whereas the cationic response to carbamylcholine appears little affected in the absence of Ca^{2+} (Fig. 6).

There are reasons to believe that the postulated intracellular redistribution of Ca^{2+} is not sufficient to account fully for the stimulation of insulin release

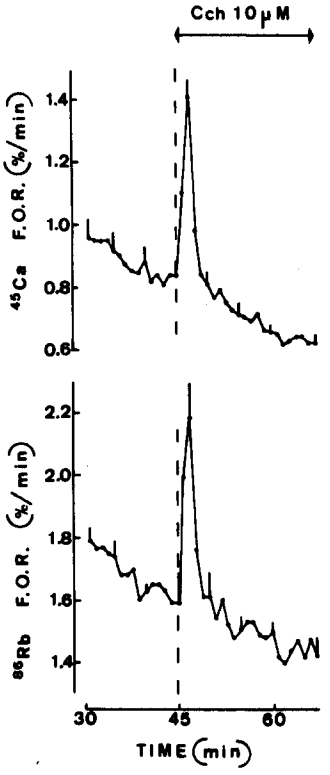

Fig. 6. Effect of carbamylcholine (10 μM), administered from min 46 to 67, upon ^{45}Ca and ^{86}Rb FOR from islets perifused in the absence of Ca^{2+} and presence of EGTA (0.5 mM) and glucose (4.4 mM). Mean values (± S.E.M.) refer to 8 (top) and 4 (bottom) individual experiments.

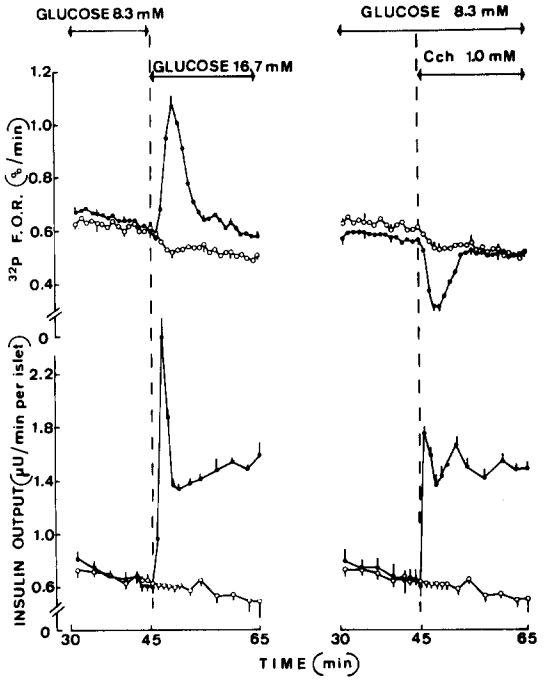

Fig. 7. Effect of an increase in D-glucose concentration from 8.3 to 16.7 mM (left) and the administration of carbamylcholine (1.0 mM) in the presence of D-glucose 8.3 mM (right) upon ^{32}P FOR and insulin release from islets perifused at normal Ca^{2+} concentration (1.0 mM). The data obtained in control experiments performed throughout in the presence of 8.3 mM D-glucose are shown as open circles. Mean values (± S.E.M., or with the range of individual variations when N = 2) refer to 2 (left), 4 (right) and 2 (control) individual experiments.

by carbamylcholine. First, the cationic response to carbamylcholine persisted whereas the secretory response is known to be abolished in the absence of extracellular Ca^{2+}. This suggests that a sufficient supply of extracellular Ca^{2+} plays at least a permissive role in the stimulation of insulin release by cholinergic agents. Second, if cholinergic agents were to cause a sustained increase in cytosolic Ca^{2+} concentration, this would be expected to coincide with stimulation of cyclic AMP production [15]. Yet, such is not the case as judged from either prior [22] or the present results. Last, the time course of the cationic and secretory responses, respectively, were not superimposable. The stimulation of ^{45}Ca FOR represented mainly an early, peak-shaped phenomenon. A modest stimulation of ^{45}Ca outflow during the late period of exposure to carbamylcholine was evidenced solely by the limited decrease in ^{45}Ca FOR recorded upon removal of the cholinergic agent. In contrast, the secretory response displayed a biphasic pattern with a sustained and elevated output of insulin during the late phase of stimulation. In the light of a recent proposal [32, 33], it is conceivable that the stimulation of insulin release by cholinergic agents involves, in addition to the postulated intracellular redistribution of Ca^{2+} and especially during the late secretory phase, activation of protein kinase C by diacylglycerol liberated through the hydrolysis of phosphoinositides.

In conclusion, the present work suggests that the stimulation by carbamylcholine of bioelectrical and secretory activity in islet cells cannot be accounted for by a decrease in K^+ conductance or increase in phosphate efflux, as observed in response to nutrient secretagogues. Instead, cholinergic agents apparently mobilize Ca^{2+} from cellular stores. Such an effect may participate in the stimulation of insulin release, but seems insufficient to account fully for the secretory response to these agents.

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