

Mechanisms of the stimulation of insulin release by oxytocin in normal mouse islets

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Oxytocin (OT) produced a dose-dependent increase in somatostatin, glucagon and insulin release by isolated mouse islets. A small effect on somatostatin release was observed with 0.1 nM-OT, but 1–10 nM-OT was required to affect A- and B-cells significantly. The effects of OT on somatostatin and glucagon release were similar in the presence of 3 mM- and 10 mM-glucose. No change in insulin release was produced by OT in 3 mM-glucose, but a stimulation was still observed in the presence of a maximally effective concentration of glucose (30 mM). The increase in insulin release produced by OT (in 15 mM-glucose) was accompanied by small accelerations of ^{86}Rb and ^{45}Ca efflux from islet cells. Omission of extracellular Ca^{2+} accentuated the effect of OT on ^{86}Rb efflux, attenuated that on ^{45}Ca efflux, and abolished that on release. OT never inhibited ^{86}Rb efflux. It did not affect the resting potential of B-cells, but slightly increased the Ca^{2+} -dependent electrical activity induced by 15 mM-glucose. OT did not affect cyclic AMP levels, but increased inositol phosphate levels in islet cells. It is suggested that the amplification of glucose-induced insulin release that OT produces is due to a stimulation of phosphoinositide metabolism, and presumably an activation of protein kinase C, rather than to a change in cyclic AMP levels or a direct action on the membrane potential. Since OT is present in the pancreas, it is possible that it exerts a neuropeptidergic control of the islet function.

INTRODUCTION

Besides its major physiological effects on the uterus and mammary gland, oxytocin (OT) exerts several metabolic actions. Changes in plasma concentrations of glucose and fatty acids have been noted after OT administration [1–4]. It has also been reported that OT increases plasma levels of insulin and glucagon in animals [5–9] and in man [10,11].

Studies *in vivo* have established that OT modulates lipolysis in adipocytes [12], increases glycogenolysis in hepatocytes [13], and directly affects hormone release by the endocrine pancreas. OT was found to increase glucagon and insulin secretion by the isolated and perfused rat pancreas [14] and glucagon secretion by isolated and incubated rat islets [15]. The mechanisms of these stimulatory effects of OT are, however, unknown. Though surprising at first sight, the possibility that OT plays a role in the control of the endocrine pancreas is supported by the recent biochemical demonstration of the presence of OT in the pancreas [16], by the immunohistochemical detection of OT in nerve terminals in the islets [17], and by the identification of OT receptors in islet cells [18].

The present study is an investigation of the mechanisms by which OT affects hormone release by normal mouse islets.

MATERIALS AND METHODS

All experiments were performed with islets of fed female NMRI mice (25–30 g), killed by decapitation. For electrophysiological experiments, a piece of pancreas was fixed in a perfusion chamber and islets were partially microdissected by hand. The membrane potential of single B-cells was continuously recorded with high-resistance microelectrodes [19]. B-cells were identified by the typical electrical activity that they display in the presence of 15 mM-glucose.

For all other experiments, islets were isolated after collagenase digestion of the pancreas. After isolation, the islets were loaded with ^{45}Ca or ^{86}Rb (used as tracer for K^+) during 90 min of incubation in 0.25 ml of medium containing 15 mM-glucose. The islets were then washed and placed in batches of 25–30 in perfusion chambers [20]. The radioactivity lost by the islets was measured in effluent fractions collected at 2 min intervals, and the fractional efflux rate was calculated for each period. A portion of each effluent fraction was taken for insulin assay. Release of insulin, glucagon and somatostatin was also measured in incubation experiments. After 60 min of preincubation in a medium containing 15 mM-glucose, batches of seven islets were incubated for 60 min in 1 ml of medium containing 3 mM- or 10 mM-glucose and supplemented with OT (0.001–100 nM). A portion of the medium was withdrawn and appropriately diluted at the end of the incubation. Insulin was measured by a double-antibody radioimmunoassay with rat insulin as standard (Novo Research Institute, Bagsvaerd, Denmark). Glucagon and somatostatin were measured by a radioimmunoassay using a dextran-coated-charcoal separation step [21].

For measurement of inositol phosphate levels, islets were first loaded with *myo*-[2- ^3H]inositol for 2 h. Free inositol and inositol phosphates were separated by anion-exchange chromatography [22]. Details of the technique have been reported recently [23]. Islet cyclic AMP concentrations were determined by radioimmunoassay with a commercially available kit (Du Pont–New England Nuclear, Boston, MA, U.S.A.) after acetylation of samples [23,24].

The basal medium used was a bicarbonate-buffered solution which contained 120 mM-NaCl, 4.8 mM-KCl, 2.5 mM- CaCl_2 , 1.2 mM- MgCl_2 and 24 mM- NaHCO_3 . It was gassed with O_2/CO_2 (47:3), had a pH of 7.4 at 37 °C, and, except for electrophysiological experiments, was supplemented with BSA (1 mg/ml).

OT was obtained from Peninsula Laboratories (Belmont, CA,

Abbreviation used: OT, oxytocin.

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U.S.A.). The same batch was used for all experiments. Forskolin was from Calbiochem-Behring (San Diego, CA, U.S.A.). Radiochemicals were purchased from The Radiochemical Centre (Amersham, Bucks., U.K.).

Electrophysiological experiments are illustrated by a recording that is representative of the indicated number of experiments. Otherwise, results are presented as means \pm S.E.M. for the indicated numbers of experiments (different animals or islet preparations) or batches of islets. The statistical significance of differences between means was assessed by comparing control and test conditions (electrophysiological experiments) by a paired *t* test, or by analysis of variance followed by a Dunnett test. Differences were considered significant at $P < 0.05$.

RESULTS

Effects of OT on hormone release by incubated islets

Somatostatin release by control islets was 2.4-fold higher in the presence of 10 mM-glucose than in the presence of 3 mM-glucose

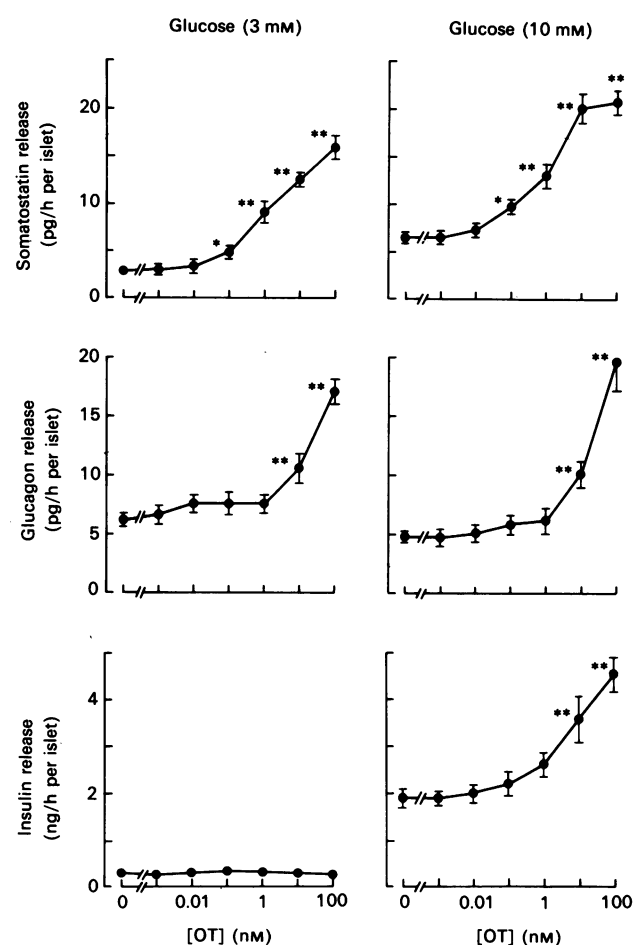


Fig. 1. Effects of various concentrations of OT on release of somatostatin, glucagon and insulin from mouse islets

Batches of seven islets were incubated for 60 min in 1 ml of medium containing 3 mM- or 10 mM-glucose, and supplemented with the indicated concentration of OT. Values are means \pm S.E.M. for 10–15 batches of islets from at least five different experiments: * $P < 0.05$ and ** $P < 0.01$ versus controls without OT.

(6.4 ± 0.3 versus 2.7 ± 0.3 pg/h per islet; $P < 0.001$). OT produced a dose-dependent increase in somatostatin release at both glucose concentrations (Fig. 1). The lowest effective concentration of OT was 0.1 nM. In 10 mM-glucose, the half-maximal response was observed at about 1 nM-OT.

Glucagon release by control islets was only marginally lower in 10 mM- than in 3 mM-glucose (4.8 ± 0.4 versus 6.2 ± 0.6 pg/h per islet; $P = 0.05$); the major inhibition (by about 60%) occurs between 0 and 3 mM-glucose. OT (10 and 100 nM) increased glucagon release, and this effect was similar at both glucose concentrations.

Insulin release by control islets was stimulated 6.3-fold by 10 mM-glucose compared with 3 mM-glucose (1.9 ± 0.2 versus 0.3 ± 0.04 ng/h per islet; $P < 0.001$). OT did not affect insulin release in 3 mM-glucose, but increased it in the presence of 10 mM-glucose. The 39% increase produced by 1 nM-OT almost reached statistical significance ($0.05 < P < 0.1$). Separate experiments showed that 1 μ M-OT was not more effective than 100 nM-OT (2.3 ± 0.4 ng/h per islet in 10 mM-glucose alone, and 4.9 ± 0.7 and 5.0 ± 0.6 ng/h per islet in the presence of 100 nM- and 1 μ M-OT respectively; means \pm S.E.M.; $n = 10$). One may thus tentatively estimate a half-maximally effective concentration of about 5 nM-OT in 10 mM-glucose. The maximal effect of OT was also observed at 100 nM in the presence of 15 mM-glucose (8.3 ± 0.6 ng/h per islet in 15 mM-glucose alone, and 16.3 ± 0.9 and 17.0 ± 1.0 ng/h per islet in the presence of 100 nM- and 1 μ M-OT respectively; means \pm S.E.M.; $n = 25$).

Effects of OT on insulin release and ionic fluxes in perfused islets

OT (100 nM) increased ^{45}Ca efflux, ^{86}Rb efflux and insulin release from islets perfused with a medium containing 15 mM-glucose and 2.5 mM- Ca^{2+} (Fig. 2). The accelerations of ^{45}Ca efflux and ^{86}Rb efflux rapidly stabilized and were thus of small amplitude, whereas the increase in insulin release did not reach a plateau until after 20 min of stimulation with OT. All these changes were reversible on withdrawal of OT.

When the medium did not contain Ca^{2+} , insulin release was not stimulated by glucose and was not increased by 100 nM-OT, which, however, still accelerated ^{45}Ca and ^{86}Rb efflux (Fig. 3). The increase in ^{45}Ca efflux was most marked during the first minutes of stimulation, but subsequently faded out. On the other hand, the increase in ^{86}Rb efflux was sustained and was clearly larger than in the presence of Ca^{2+} .

^{86}Rb efflux was also measured in the presence of 3 mM-glucose. The slow decrease in the efflux rate that occurs under these conditions was unaffected by 100 nM-OT: $2.40 \pm 0.05\%$ /min at 40 min, $2.04 \pm 0.02\%$ /min at 70 min in control islets, and $2.08 \pm 0.07\%$ /min in islets stimulated by 100 nM-OT since 40 min ($n = 5$). OT was also without effect on insulin release by these islets perfused with a medium containing only 3 mM-glucose (results not shown).

In the presence of a maximally effective concentration of glucose (30 mM), the rate of insulin release averaged 336 ± 53 pg/min per islet. It increased to 634 ± 75 pg/min per islet ($n = 4$) after 30 min of stimulation with 100 nM-OT.

Effects of OT on the membrane potential of B-cells

The resting membrane potential of B-cells perfused with a medium containing 3 mM-glucose was not affected by 100 nM-OT (-60 ± 4 mV without OT versus -61 ± 3 mV with OT; $n = 3$).

In the presence of 15 mM-glucose, B-cells were depolarized and exhibited a rhythmic electrical activity, consisting of slow waves of the membrane potential with Ca^{2+} spikes superimposed on the plateau (Fig. 4). OT (100 nM) had little effect on this electrical

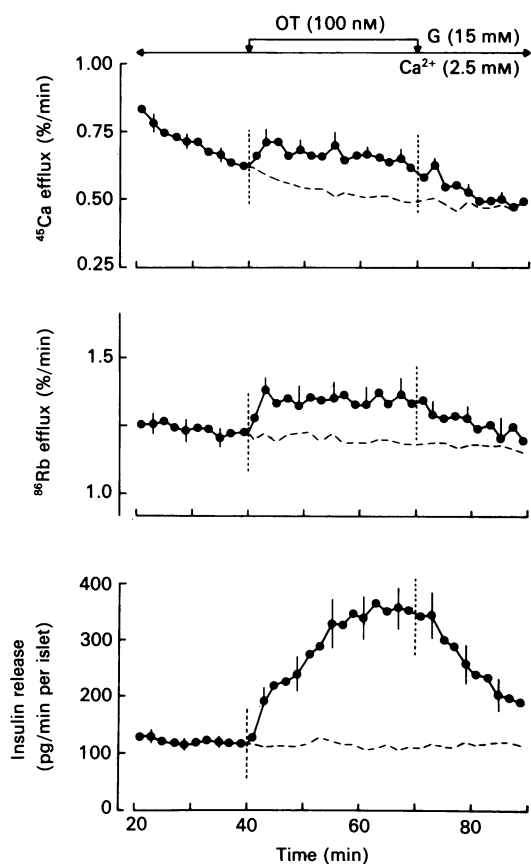


Fig. 2. Effects of OT on ^{45}Ca efflux, ^{86}Rb efflux and insulin release from mouse islets perfused with a medium containing 15 mM-glucose (G) and 2.5 mM- Ca^{2+} .

OT (100 nM) was added between 40 and 70 min. Control experiments without OT are shown by the broken lines. Values are means \pm S.E.M. for four to six experiments.

activity. The lower panels summarize the quantification of the effects of OT after 6 or 16 min of application. The duration of the slow waves with spikes was not modified by OT, but that of the intervals slightly decreased. The frequency of the slow waves slightly increased. The net effect on the overall electrical activity was a small increase in the plateau phase (percentage of time with spike activity) (Fig. 4).

Effects of OT on inositol phosphate and cyclic AMP levels

Inositol phosphate levels were measured in islets which were preincubated with *myo*-[2- ^3H]inositol to label their phosphoinositides, and then stimulated by 100 nM-OT in a medium containing 5 mM-LiCl (Table 1). OT significantly increased the levels of InsP_1 , InsP_2 and InsP_3 .

In contrast with forskolin, 100 nM-OT did not increase islet cyclic AMP levels (Table 1), but both agents potentiated insulin release.

DISCUSSION

The present study establishes that OT stimulates somatostatin release, confirms that OT increases glucagon and insulin release, and demonstrates that a stimulation of phosphoinositide meta-

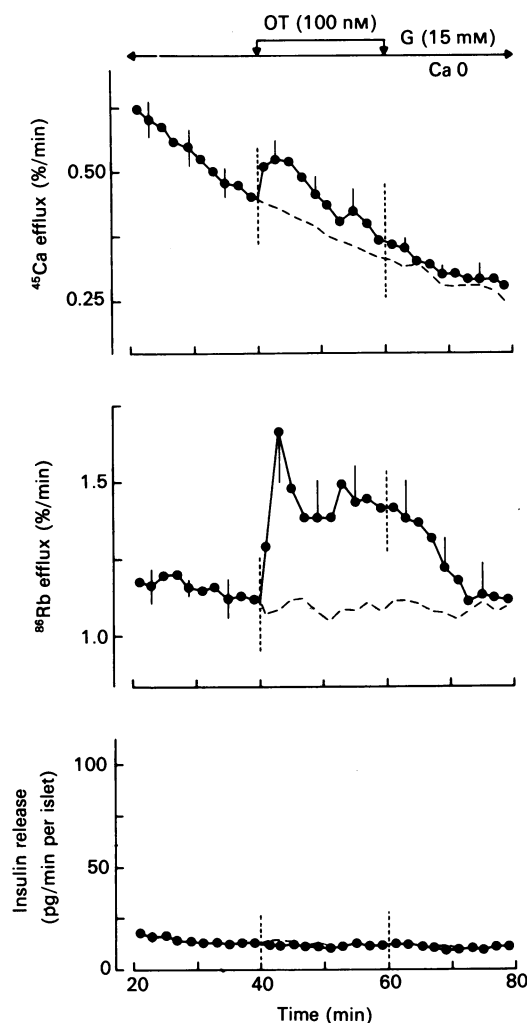


Fig. 3. Effects of OT on ^{45}Ca efflux, ^{86}Rb efflux and insulin release from mouse islets perfused with a Ca^{2+} -free medium containing 15 mM-glucose (G).

OT (100 nM) was added between 40 and 60 min. Control experiments without OT are shown by the broken lines. Values are means \pm S.E.M. for four to five experiments.

bolism rather than a change in ionic fluxes, membrane potential or cyclic AMP underlies the effects of OT in B-cells. The interpretation of the results is based on the widely accepted assumption that biochemical or ionic changes measured in whole islets are representative of the changes occurring in B-cells, which make up 70–80% of the islet population.

In experiments using isolated rat islets or the perfused rat pancreas, Dunning *et al.* observed that OT stimulated glucagon release, but had little effect on insulin release [14,15]. This difference in the sensitivity of A- and B-cells to OT was not observed in the present study. Of the three islet cell types, D-cells appear to be the most sensitive to OT in the mouse, but the differences are not very marked. In the presence of 10 mM-glucose, the estimated half-maximally effective concentrations of OT were 1 nM (D-cells) and 5 nM (B-cells). These values correspond well to the affinity of OT receptors (K_d 1–5 nM) in other tissues [25].

The effectiveness of OT on somatostatin release in 3 mM-glucose may seem surprising. It should, however, be recalled that the threshold glucose concentration for stimulation of D-cells is lower than that for stimulation of B-cells [26,27]. In our system

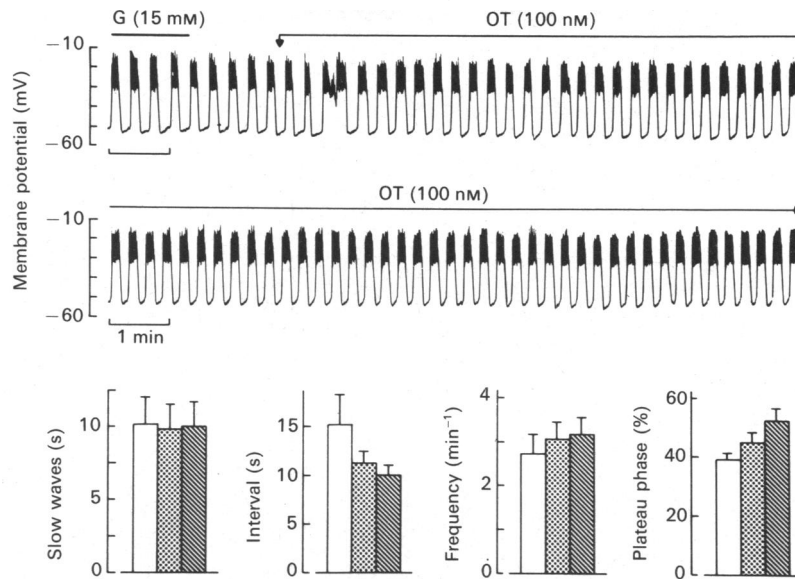


Fig. 4. Effects of OT on the membrane potential of mouse pancreatic B-cells perfused with a medium containing 15 mM-glucose (G) and 2.5 mM-Ca²⁺

OT (100 nM) was added as indicated by the arrows. The lower recording is the direct continuation of the upper one. The lower panels show the quantitative changes (means \pm S.E.M.) in electrical activity in four similar experiments (different mice). All slow waves and intervals were measured during the last 4 min of perfusion with glucose alone (\square), and during the periods 6–10 min (\square) and 16–20 min (\blacksquare) of the stimulation with 100 nM-OT. The plateau phase is the percentage of time spent at the plateau potential with spike activity.

Table 1. Effects of oxytocin on inositol phosphate and cyclic AMP levels in mouse islets

For measurement of inositol phosphate levels, the islets were first preincubated for 120 min in a medium containing 15 mM-glucose and supplemented with *myo*-[³H]inositol. They were then washed and subdivided in batches of 40–50. For measurements of cyclic AMP levels, the islets were first preincubated for 60 min in a medium containing 15 mM-glucose. They were then subdivided in batches of 10. Each batch of islets was eventually incubated for 60 min in a medium containing 15 mM-glucose. When tested, oxytocin and forskolin were added for the last 30 min of incubation only. In the experiments designed to measure islet cyclic AMP levels, the incubation medium was saved for measurement of insulin release. Values are means \pm S.E.M. for 5 batches of islets (inositol phosphates), or for 12 or 18 batches of islets (cyclic AMP): **P* < 0.01 versus controls (by analysis of variance followed by Dunnett's test).

| Experimental conditions | Content (d.p.m./islet) | | | Cyclic AMP (fmol/islet) | Insulin release (ng/h per islet) |
|-------------------------|------------------------|-------------------|-------------------|-------------------------|----------------------------------|
| | InsP ₁ | InsP ₂ | InsP ₃ | | |
| Controls | 69 \pm 4 | 42 \pm 4 | 32 \pm 1 | 22.0 \pm 1.2 | 6.5 \pm 0.4 |
| Oxytocin (100 nM) | 411 \pm 24* | 60 \pm 4* | 39 \pm 3* | 22.3 \pm 1.2 | 9.3 \pm 0.4* |
| Forskolin (100 nM) | — | — | — | 52.5 \pm 3.6* | 13.5 \pm 0.9* |

3 mM-glucose slightly increases somatostatin release compared with zero glucose, whereas insulin release is unaffected (Z.-Y. Gao & J.-C. Henquin, unpublished work).

The maximum concentrations of somatostatin and glucagon reached in the medium at the end of the incubations with 100 nM-OT were between 0.01 and 0.1 nM. These concentrations are too low to affect insulin release by intact islets [28]. It is thus unlikely that the stimulation of somatostatin and glucagon release by OT markedly influences the B-cell response, except if substantially higher concentrations exist within the islets.

K⁺ channels of the B-cell membrane, in particular the ATP-sensitive K⁺ channels, are a major target for agents modulating insulin release [29,30]. Their closure, e.g. by glucose or sulphonylureas, underlies the depolarization that activates Ca²⁺ channels and eventually triggers release. Several arguments make it possible to exclude the hypothesis that the effects of OT result from a closure of these channels. Firstly, OT neither inhibited ⁸⁶Rb efflux from islet cells nor depolarized the B-cell membrane in the presence of 3 mM-glucose. Secondly, OT accelerated ⁸⁶Rb efflux from islets perfused with 15 mM-glucose, in both the

absence and the presence of extracellular Ca²⁺, whereas, at this glucose concentration, agents which block ATP-sensitive K⁺ channels would increase ⁸⁶Rb efflux in the presence of Ca²⁺ but decrease it in the absence of Ca²⁺ [31,32]. Thirdly, the small increase in electrical activity produced by OT in B-cells perfused with a medium containing 15 mM-glucose was not characterized by a lengthening of the slow waves like that produced by higher concentrations of glucose or by sulphonylureas [33]. On the other hand, the small increase in slow-wave frequency secondary to the shortening of the intervals, which OT produced, closely resembles the effect of various activators of protein kinase C [34,35]. A further similarity is the paradoxically larger acceleration of ⁸⁶Rb efflux in the absence than in the presence of extracellular Ca²⁺. A conservative conclusion is thus that the small effects of OT on the membrane potential are mediated by an activation of protein kinase C.

The results show that OT both increased Ca²⁺ influx and mobilized intracellular Ca²⁺ in B-cells, but that the potentiation of insulin release required the presence of extracellular Ca²⁺. That OT causes a small increase in Ca²⁺ influx is indicated by two

observations. Firstly, OT accelerated ^{45}Ca efflux more markedly in the presence than in the absence of extracellular Ca^{2+} . Secondly, glucose-induced electrical activity, which reflects Ca^{2+} influx in B-cells [32], was slightly augmented by OT. It is thus possible that a small increase in Ca^{2+} influx contributes to the effects of OT on insulin release, but there is no doubt that other mechanisms are involved. The combination of 15 mM-glucose and OT increased insulin release to a similar rate as in the presence of 30 mM-glucose alone, although the plateau phase of electrical activity was still below 60%, compared with 100% in 30 mM-glucose [33].

The small increase in ^{45}Ca efflux that OT consistently caused in islets perfused with a Ca^{2+} -free medium indicates that OT mobilizes intracellular Ca^{2+} in B-cells. No insulin release occurred simultaneously, probably because this mobilization was of too small magnitude. In experiments using various concentrations of acetylcholine, it was observed that insulin release in the absence of Ca^{2+} increases only when Ca^{2+} mobilization exceeds a certain threshold [36]. This threshold, attained with 10 μM -acetylcholine, was not reached during the stimulation by OT, the effect of which is similar to that of 1 μM -acetylcholine.

Stimulation of phosphoinositide metabolism by OT has been observed in the classical target tissues of the hormone, the uterus [37–39] and the mammary gland [40], and in adipocytes [41]. That OT also stimulated phosphoinositide turnover in B-cells is indicated by the increases in the levels of labelled InsP_1 , InsP_2 and InsP_3 that it produced in islets prelabelled with *myo*- ^3H inositol. Although the isomer of InsP_3 cannot be identified with the technique used [22], the results are compatible with the hypothesis that the mobilization of intracellular Ca^{2+} is due to the production of $\text{Ins}(1,4,5)\text{P}_3$. It also seems reasonable to assume that diacylglycerol was produced concomitantly with the production of inositol phosphates [42], and that the activation of protein kinase C ensued. That an activation of protein kinase C participates in the increase in insulin release brought about by OT is in keeping with the lack of effect of OT in low glucose, with the persistence of its effect in the presence of a maximally effective concentration of the sugar, and with the requirement of extracellular Ca^{2+} [34,36,43].

OT has been reported to increase cyclic AMP levels in LLC-PK₁ kidney epithelial cells, an effect possibly mediated by V_2 -vasopressin receptors [44]. No effect of OT was observed on cyclic AMP levels in islet cells. Our data therefore support the concept that OT receptors are not coupled to adenylate cyclase.

In conclusion, OT potently amplifies glucose-induced insulin release in normal mouse islets. This effect appears to involve a stimulation of phosphoinositide metabolism with its consequences on Ca^{2+} movements and on activation of protein kinase C. The results also show that OT increases somatostatin and glucagon release, but cannot prove that this increase is underlain by the same mechanisms. This hypothesis would, however, be compatible with the reports that activation of protein kinase C by phorbol esters induces glucagon release by pancreatic A-cells [45] and somatostatin release by gastric D-cells [46]. The effects of OT on the endocrine pancreas resemble those of vasopressin, which was recently shown to amplify insulin release by stimulating phosphoinositide metabolism in B-cells [23,47]. This similarity raises the question of whether both peptides exert their action by activating the same or distinct receptors [48]. We note, however, that both OT and [arginine]-vasopressin have been identified in the pancreas of several species [17] and that they might thus have a local neuropeptidergic function.

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