Parathyroid Hormone Controls the Size of the Intracellular Ca\(^{2+}\) Stores Available to Receptors Linked to Inositol Trisphosphate Formation*

(Received for publication, August 17, 1999, and in revised form, November 5, 1999)

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In HEK 293 cells stably expressing type 1 parathyroid (PTH) receptors, PTH stimulated release of intracellular \(\text{Ca}^{2+}\) stores in only 27% of cells, whereas 96% of cells responded to carbachol. However, in almost all cells PTH potentiated the response to carbachol by about 3-fold. Responses to carbachol did not desensitize, but only the first challenge in \(\text{Ca}^{2+}\)-free medium caused an increase in \([\text{Ca}^{2+}]_i\), indicating that the carbachol-sensitive \(\text{Ca}^{2+}\) stores had been emptied. Subsequent addition of PTH also increased \([\text{Ca}^{2+}]_i\), but when it was followed by carbachol there was a substantial increase in \([\text{Ca}^{2+}]_i\). A similar potentiation was observed between ATP and PTH but not between carbachol and ATP. Intracellular heparin inhibited responses to carbachol and PTH, and pretreatment with ATP and carbachol abolished responses to PTH, suggesting that the effects of PTH involve inositol trisphosphate (IP\(_3\)) receptors. PTH neither stimulated detectable IP\(_3\) formation nor affected the amount formed in response to ATP or carbachol. PTH stimulated cyclic AMP formation, but this was not the means whereby PTH potentiated \(\text{Ca}^{2+}\) signals. We suggest that PTH may regulate \(\text{Ca}^{2+}\) mobilization by facilitating translocation of \(\text{Ca}^{2+}\) between discrete intracellular stores and that it thereby regulates the size of the \(\text{Ca}^{2+}\) pool available to receptors linked to IP\(_3\) formation.

Parathyroid hormone (PTH) plays a central role in plasma \(\text{Ca}^{2+}\) homeostasis, and many tissues that are not involved in \(\text{Ca}^{2+}\) regulation also express PTH receptors (1). Two PTH receptor subtypes (types 1 and 2) have been identified; their cDNA sequences share 70% similarity (2–5) and identify them as members of a subfamily of \(G\) protein-coupled receptors to which the receptors for calcitonin, secretin, ACTH, and glucagon also belong.

It has long been accepted that PTH stimulates an increase in both intracellular cyclic AMP and \([\text{Ca}^{2+}]_i\), in many cell types (6, 7). The ability of PTH to activate two signaling pathways was generally assumed to result from its interaction with the two receptor subtypes, an assumption that gained support from the observation that the two pathways could be differentially stimulated by truncated forms of PTH (8). More recently, expression of recombinant receptors has established that type 1 (3, 9, 10) and type 2 (11) PTH receptors are each alone capable of stimulating increases in both \([\text{Ca}^{2+}]_i\), and intracellular cyclic AMP. Other members of the family of receptors to which the PTH receptor belongs share this ability to independently stimulate cyclic AMP formation and an increase in \([\text{Ca}^{2+}]_i\) (3, 12–14).

In some cells, the increase in \([\text{Ca}^{2+}]_i\), evoked by PTH is mediated largely by effects of cyclic AMP on \(\text{Ca}^{2+}\) entry (7), but in osteoblasts and kidney cells (6) and cells expressing recombinant type 1 (3, 9, 10) or type 2 (11) PTH receptors, PTH also stimulates release of intracellular \(\text{Ca}^{2+}\) stores. The means whereby PTH causes such \(\text{Ca}^{2+}\) mobilization is unclear. In some cells, PTH stimulates formation of inositol 1,4,5-trisphosphate (IP\(_3\)) (3, 6), which presumably then causes \(\text{Ca}^{2+}\) mobilization via IP\(_3\) receptors. In other situations, PTH and agonists that stimulate IP\(_3\) formation appear to release \(\text{Ca}^{2+}\) from different intracellular stores (9, 15), and PTH-stimulated \(\text{Ca}^{2+}\) mobilization occurs without detectable formation of IP\(_3\) (10, 15) and in the presence of antagonists of IP\(_3\) receptors (9, 10). Furthermore, PTH-evoked \(\text{Ca}^{2+}\) release appears not to be mediated by ryanodine receptors (9) or by either cyclic ADP-ribose or NAADP\(^{7+}\) (9), both of which have been shown to stimulate \(\text{Ca}^{2+}\) release in other cells (16). In short, the ability of PTH to stimulate \(\text{Ca}^{2+}\) mobilization cannot easily be explained by the properties of known intracellular signaling pathways.

In the present study, we examine the mechanisms underlying the effects of PTH on intracellular \(\text{Ca}^{2+}\) stores in human embryonic kidney 293 cells stably expressing type 1 PTH receptors (HEK/PTH-R1 cells).

EXPERIMENTAL PROCEDURES

Materials—The full-length cDNA encoding the human type 1 PTH receptor in the vector, pcDNA\(\varnothing\)nes, was a gift from Dr. K. Seuwen (Basel, Switzerland) (5). HEK 293 cells were from the European Collection of Animal Cell Cultures (Porton Down, UK). Human PTH (1–34), Rp-8-Br-cAMPs, ionomycin, Xestospongin A, wortmannin, and H89 were from Calbiochem (Nottingham, UK). Fura-2AM and Cascade Blue were from Molecular Probes (Leiden, The Netherlands). Forskolin, heparin, and poly-I-lysine were from Sigma. Tissue culture media, G-418, LipofectAMINE, and OptiMEM were from Life Technologies, Inc. The Biotrak cyclic AMP assay kit and \(\text{n}-\text{myo-[2-3H]}\) inositol were from Amersham Pharmacia Biotech.

Transfection and Cell Culture—HEK 293 cells were transfected with the expression vector, pRezex-2, containing the complete coding sequence of the human type 1 PTH receptor (5). Cells expressing PTH receptors were selected by screening for those that responded to PTH with an increase in \([\text{Ca}^{2+}]_i\) (see below). Cells stably transfected with the PTH receptor (HEK/PTH-R1) were grown in Dulbecco's modified Eagle's medium/Ham's F-12 medium supplemented with fetal calf serum (10%), glutamine (2 mM), and G-418 (800 \(\mu\)g/ml). Responses to PTH were maintained during at least 40 passages.
RESULTS AND DISCUSSION

Ca\textsuperscript{2+} Mobilization Evoked by Type 1 PTH Receptors—Stimulation of the endogenous muscarinic receptors of HEK 293 cells with carbachol (100 \mu M) evoked an increase in [Ca\textsuperscript{2+}]i irrespective of whether the cells had been transfected with PTH receptors (Fig. 1, A–C). PTH (1 \mu M), however, stimulated an increase in [Ca\textsuperscript{2+}]i only in HEK/PTH-R1 cells, and, in keeping with previous reports comparing PTH with agonists that stimulate IP\textsubscript{3} formation (9, 15, 19), the maximal response to PTH was only 21 ± 5% (n = 7) of that evoked by carbachol (Fig. 1B). In one previous study (19) but not in others (10, 11, 20, 21), it was claimed that HEK 293 cells express type 1 PTH receptors. Our results are consistent with most previous work in demonstrating that PTH evokes Ca\textsuperscript{2+} mobilization in HEK 293 cells only after transfection with type 1 PTH receptors (Fig. 1).

Although the sustained phase of the Ca\textsuperscript{2+} signal evoked by carbachol and the peak amplitudes of the responses evoked by PTH or carbachol were reduced by removal of extracellular Ca\textsuperscript{2+} (Fig. 1, B and C), both agonists consistently evoked an increase in [Ca\textsuperscript{2+}]i in the absence of extracellular Ca\textsuperscript{2+} (Fig. 1C), and responses to both were abolished after pretreatment with thapsigargin in Ca\textsuperscript{2+}-free ECM (not shown). We conclude that both PTH and carbachol stimulate release of intracellular Ca\textsuperscript{2+} stores. Subsequent experiments addressed the mechanisms responsible for this Ca\textsuperscript{2+} mobilization.

Single cell analyses of HEK/PTH-R1 cells revealed that whereas 96 ± 5% (n = 12 coverslips) of cells responded to carbachol (100 \mu M) with an increase in [Ca\textsuperscript{2+}]i, only 27 ± 20% of cells responded to PTH (100 \mu M). In those cells that responded to PTH, the peak increase in [Ca\textsuperscript{2+}]i was 84 ± 16% (n = 11) of that evoked by carbachol in the same cells (Fig. 1D). Single cell analyses of HEK/PTH-R1 cells were used for all subsequent experiments (see “Experimental Procedures”).

The magnitude of the responses to maximal concentrations of carbachol and PTH varied between experiments, although the relative amplitudes of the responses to the two stimuli were maintained. For each cell, we therefore expressed all responses as percentages of the response evoked by carbachol (100 \mu M) in normal ECM at the beginning of the experiment. After stimulation with carbachol in normal ECM, cells were allowed to recover for 5 min. Further stimulation then evoked indistin-

Measurement of [Ca\textsuperscript{2+}]i—HEK/PTH-R1 cells were plated (2.5 × 10\textsuperscript{5} cells/ml) onto glass coverslips coated with poly-1-lysine and grown for at least 2 days. Confluent cells were loaded with Fura-2 by incubation with Fura-2AM (1 \mu M, 60 min) dissolved in extracellular medium (ECM; 130 mM NaCl, 5.4 mM KCl, 0.8 mM Na\textsubscript{2}HPO\textsubscript{4}, 1.8 mM CaCl\textsubscript{2}, 0.9 mM MgSO\textsubscript{4}, 10 mM glucose, 20 mM Hepes, pH 7.4) at 20 °C for 45 min. The cells were then washed, and after 30 min in ECM, they were used for experiments. Previous experiments established that with this loading protocol, >95% of the Fura-2 fluorescence came from Fura-2 that was both cytosolic and hydrolyzed to its Ca\textsuperscript{2+}-sensitive form (17). Single cell fluorescence imaging at 21 °C using IonVision software, corrections for background fluorescence, and calibration of fluorescence ratios (R\textsubscript{340/380}) to [Ca\textsuperscript{2+}]i, were performed as described previously (17). For microinjection with heparin, Fura-2-loaded cells bathed in nominally Ca\textsuperscript{2+}-free ECM were injected with medium (27 mM K\textsubscript{2}HPO\textsubscript{4}, 8 mM Na\textsubscript{2}HPO\textsubscript{4}, 26 mM KH\textsubscript{2}PO\textsubscript{4}, pH 7.3) containing 10 mg/ml heparin and 1 mg/ml 6-mercaptoethanol as a marker for microinjected cells (18). After 30 min, cells were restored to Ca\textsuperscript{2+}-containing ECM prior to measurement of [Ca\textsuperscript{2+}]i.

Measurement of Cyclic AMP Production—Confluent cultures of HEK/PTH-R1 cells were plated (2.5 × 10\textsuperscript{5} cells/ml) onto poly-L-lysine-coated glass coverslips. For most experiments, responses from 25–30 single cells (106) were averaged. Statistical analyses were applied to the average results derived from independent measurements from several coverslips, where the sample size refers to the number of coverslips. Concentration-effect relationships were fitted to four-parameter logistic equations using least squares curve-fitting routines (Kaleidagraph, Synergy Software).

![Figure 1](image-url)
Cross-potentiation of the Ca\textsuperscript{2+} mobilization evoked by PTH and carbachol in HEK/PTH-R1 cells. A and B, cells were first stimulated with carbachol (CCh) (100 μM) in Ca\textsuperscript{2+}-containing ECM (reference response) before removal of extracellular Ca\textsuperscript{2+} and successive stimulation with PTH (100 nM) and then carbachol (100 μM) (A) or carbachol and then PTH (B). Traces show average responses of about 40 cells on a single coverslip and are typical of recordings from at least eight coverslips. C, results from experiments similar to those in A and B are summarized with the peak rise in [Ca\textsuperscript{2+}] (percentage of that initially evoked by carbachol in Ca\textsuperscript{2+}-containing ECM) shown after stimulation with carbachol or PTH alone or in rapid succession. Open bars denote results obtained in Ca\textsuperscript{2+}-free ECM, and filled bars show responses in Ca\textsuperscript{2+}-containing ECM. Results are means ± S.E. of 8–13 independent experiments.

guishable responses; the peak increases in [Ca\textsuperscript{2+}], were 93 ± 6% (n = 3) and 94 ± 8% (n = 3) of the initial response after the second and third stimulation with carbachol, indicating that responses to carbachol do not desensitize. In subsequent experiments, changes in [Ca\textsuperscript{2+}], are expressed relative to the initial carbachol response recorded in normal ECM.

Cross-potentiation of [Ca\textsuperscript{2+}], Signals by Carbachol and PTH—PTH (100 nM) alone stimulated Ca\textsuperscript{2+} mobilization from only a fraction (27 ± 20%) of HEK/PTH-R1 cells, but it caused an increase in [Ca\textsuperscript{2+}], in most cells (86 ± 12%, n = 12 coverslips) when added immediately after carbachol (100 μM) (Fig. 2B). From the average responses of each cell in the field, the peak rise in [Ca\textsuperscript{2+}], evoked by PTH after carbachol was more than 10-fold greater than that evoked by PTH alone (160 ± 10%, n = 13; and 12 ± 7%, n = 11, respectively) (Fig. 2C). The interaction between PTH and carbachol was also evident when cells were first stimulated with PTH and then with carbachol; the peak rise in [Ca\textsuperscript{2+}], evoked by carbachol alone was 71 ± 8%

Because most HEK/PTH-R1 cells respond to PTH after carbachol pretreatment, whereas untransfected cells are unresponsive, we conclude that even though few HEK/PTH-R1 cells respond directly to PTH alone, most express functional PTH receptors. Carbachol unmasks a latent ability of PTH to stimulate release of intracellular Ca\textsuperscript{2+} stores, and PTH increases by 2–3 fold the Ca\textsuperscript{2+} mobilization evoked by carbachol, which alone stimulates Ca\textsuperscript{2+} mobilization in all cells. Potentiation of the responses to carbachol by PTH occurred despite [Ca\textsuperscript{2+}], having returned to its basal level (Fig. 2A), indicating that an increase in [Ca\textsuperscript{2+}], is not directly responsible for the effect.

**PTH Allows Carbachol to Recruit Additional Ca\textsuperscript{2+} Stores**—In Ca\textsuperscript{2+}-free ECM, carbachol caused a transient increase in [Ca\textsuperscript{2+}], in HEK/PTH-R1 cells, but subsequent challenges with carbachol failed to increase [Ca\textsuperscript{2+}], consistent with the first stimulation having fully emptied the carbachol-sensitive Ca\textsuperscript{2+} stores (Fig. 4A). PTH alone also failed to increase

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**FIG. 2. Cross-potentiation of the Ca\textsuperscript{2+} mobilization evoked by PTH and carbachol in HEK/PTH-R1 cells.** A, cells in Ca\textsuperscript{2+}-free ECM were stimulated with the indicated concentrations of carbachol alone (A) or after pretreatment with PTH (100 nM, 2 min) (B). Results (means ± S.E., n = 3) show the peak rise in [Ca\textsuperscript{2+}], expressed relative to the reference response to carbachol (CCh). B, cells in Ca\textsuperscript{2+}-free ECM were stimulated with carbachol (100 μM, 1 min) before addition of the indicated concentrations of PTH. The peak rises in [Ca\textsuperscript{2+}], detected after addition of PTH are shown (means ± S.E., n = 3) relative to the reference response to carbachol.

**FIG. 3. Effects of PTH on carbachol-evoked Ca\textsuperscript{2+} mobilization in HEK/PTH-R1 cells.** A, cells in Ca\textsuperscript{2+}-free ECM were stimulated with the indicated concentrations of carbachol alone (A) or after pretreatment with PTH (100 nM, 2 min) (B). Results (means ± S.E., n = 3) show the peak rise in [Ca\textsuperscript{2+}], expressed relative to the reference response to carbachol (CCh). B, cells in Ca\textsuperscript{2+}-free ECM were stimulated with carbachol (100 μM, 1 min) before addition of the indicated concentrations of PTH. The peak rises in [Ca\textsuperscript{2+}], detected after addition of PTH are shown (means ± S.E., n = 3) relative to the reference response to carbachol.
[Ca$^{2+}]_i$, when added 5 min after carbachol had been removed (Fig. 4A). However, a further challenge with carbachol after addition of PTH evoked a large transient increase in [Ca$^{2+}]_i$, equivalent to 135 ± 15% (n = 3) of a standard response to carbachol (Fig. 4A). We conclude that PTH allows carbachol to release intracellular Ca$^{2+}$ stores that would otherwise be insensitive to carbachol. Similar results were obtained when the order of addition of carbachol and PTH was reversed. PTH caused a substantial mobilization of intracellular Ca$^{2+}$ stores when added immediately after carbachol has been removed, even though the carbachol itself evoked no increase in [Ca$^{2+}]_i$, because the carbachol-sensitive Ca$^{2+}$ stores had already been emptied by prior stimulation (Fig. 4B).

**Cross-potentiation of [Ca$^{2+}]_i$, Signals by ATP and PTH—**ATP also stimulates IP$_3$ formation and Ca$^{2+}$ mobilization in HEK/PTH-R1 cells; the Ca$^{2+}$ mobilization evoked by 10 μM ATP was 74 ± 5% (n = 7) of the standard carbachol response (Fig. 5A). Although only 71 ± 7% (n = 6) of cells responded to ATP, in the responsive cells the synergistic interaction with PTH was again evident. PTH added immediately after removal of ATP caused a substantial increase in [Ca$^{2+}]_i$; the response to PTH was 160 ± 30% (n = 4) of the standard carbachol response (Fig. 5A). When PTH was added first, the increase in [Ca$^{2+}]_i$, evoked by subsequent addition of ATP was increased to 159 ± 28% (n = 3) of the standard carbachol response (Fig. 5B). There was no synergistic interaction between ATP and carbachol (not shown). Carbachol did not increase the fraction of cells that responded to ATP (73 ± 11% before carbachol, and 74 ± 4% after carbachol, n = 4), and in cells that responded to both stimuli, the increase in [Ca$^{2+}]_i$, evoked by combined application of carbachol and ATP (198 ± 5%) was similar to the sum of the responses evoked by either agonist alone (71 ± 5 and 97 ± 35% for carbachol and ATP, respectively).

**Mechanism of Action of PTH: the Role of IP$_3$ Receptors—**Responses to IP$_3$ are positively cooperative (22). PTH might therefore have potentiated responses to carbachol and ATP by either directly stimulating formation of a subthreshold level of IP$_3$ or by increasing the amount of IP$_3$ made in response to ATP (not shown). Carbachol did not affect the amount of IP$_3$ made in response to ATP or carbachol. Neither explanation seems likely because PTH (10 μM) neither directly stimulated detectable IP$_3$ formation (100 ± 23% of control, n = 6) nor affected the amount made in response to ATP (174 ± 23 and 186 ± 14% in the absence and presence of PTH) or carbachol (246 ± 31 and 266 ± 34%). Ca$^{2+}$ stimulates IP$_3$ receptors (22), but this cannot explain the ability of PTH to potentiate responses to carbachol and ATP because the potentiation occurred without an increase in [Ca$^{2+}]_i$, (Figs. 2, 4, and 5).

Xestospongin, which has been shown to block cerebellar IP$_3$ receptors (23), proved not to be useful in resolving the involvement of IP$_3$ receptors in the response to PTH. A range of
concentrations (10–50 μM) and preincubation periods (10–40 min) failed to separate direct effects of Xestospongin on the emptying of intracellular Ca^{2+} stores from inhibition of IP_3 receptors (not shown) (18). Two lines of evidence are, however, consistent with IP_3 receptors mediating the enhanced Ca^{2+} release evoked by ATP or carbachol with PTH. First, and in contrast with a previous report (10), microinjected heparin (10 mg/ml in the injection pipette) was similarly effective in blocking both the PTH- and carbachol-evoked increase in [Ca^{2+}]_i (Fig. 6). Second, after combined application of ATP and carbachol to release the IP_3-sensitive Ca^{2+} stores, the subsequent response to PTH was almost abolished (down from 160 ± 10 to 25 ± 22%, n = 7).

A plausible explanation for these observations is that PTH generates a signal that causes IP_3 receptors to become more sensitive to IP_3. The increase in [Ca^{2+}]_i evoked by PTH alone in some cells might then result from a sufficient basal level of IP_3 to cause Ca^{2+} mobilization from sensitized IP_3 receptors. The effects of PTH were persistent; even 10 min after removal of PTH, enhanced increases in [Ca^{2+}]_i were observed after carbachol addition (not shown); and this presumably reflects the slow dissociation of PTH from its high affinity receptor (3). However, the ability of carbachol to enhance the rise in [Ca^{2+}]_i evoked by PTH was short-lived and reversed within the ~10 s taken for the three washes required to completely remove the carbachol-containing medium, in keeping with the relatively low affinity of muscarinic receptors for carbachol and the rapid metabolism of IP_3.

Cyclic AMP Does Not Mediate the Effects of PTH on Ca^{2+} Mobilization—PTH stimulates adenylyl cyclase activity in HEK 293 cells with an EC_{50} of 20 ± 7 nM (n = 3; not shown); cyclic AMP might therefore have mediated the increases in [Ca^{2+}]_i evoked by PTH. Cyclic AMP-dependent protein kinase (PKA) phosphorylates IP_3 receptors, and, although the functional consequences differ between cell types, in hepatocytes, which like HEK 293 cells express largely type 2 IP_3 receptors (24), phosphorylation increases the sensitivity of IP_3 receptors to IP_3 (25). Forskolin was previously shown not to mimic the effects of activating type 1 PTH receptors on [Ca^{2+}]_i (10), but forskolin (5 μM) alone only very modestly increased intracellular cyclic AMP (from 0.53 ± 0.05 to 0.91 ± 0.13 pmol/well, n = 3) relative to the increase evoked by PTH (to 3.04 ± 0.05 pmol/well). Similarly modest effects of forskolin on the endogenous adenylyl cyclase activity of HEK 293 cells has been reported previously (26). However, several additional lines of evidence demonstrate that neither cyclic AMP nor PKA mediate the effects of PTH on intracellular Ca^{2+} stores. First, pretreatment with 8-bromo cyclic AMP (50 μM, 5 min) reduced the Ca^{2+} mobilization evoked by carbachol (Fig. 7A). Second, pretreatment of HEK/PTH-R1 cells with either of two inhibitors of
Fig. 8. A possible mechanism for the effects of PTH on intracellular Ca\(^{2+}\) stores. The type 1 PTH receptor, via a mechanism that does not require cyclic AMP or PI-3-kinase, is proposed to allow IP\(_3\)-sensitive Ca\(^{2+}\) stores to become linked to those lacking IP\(_3\) receptors. PTH thereby increases the size of the intracellular Ca\(^{2+}\) stores available to receptors (for ATP and carbachol (CCh)) that stimulate IP\(_3\) formation.

PKA, Rp-8-Br-cAMPS (20 μM, 15 min) (27) or H89 (5 μM, 10 min) (28), affected neither the response to PTH alone nor its ability to potentiate the response to carbachol (Fig. 7B). That these inhibitors had effectively blocked activation of PKA is confirmed by their ability to reverse the inhibition of carbachol-evoked Ca\(^{2+}\) mobilization caused by 8-bromo-cAMP (Fig. 7A). Third, 3-isobutyl-1-methyloxanthine, which by inhibiting cyclic AMP degradation would be expected to potentiate responses to PTH if they were mediated by cyclic AMP, had the opposite effect. Pretreatment with 3-isobutyl-1-methyloxanthine (1 mM, 20 min) abolished the potentiation of carbachol responses by either submaximal (6 nM) or maximal (100 nM) concentrations of PTH (not shown). We conclude that the ability of PTH to potentiate carbachol-evoked Ca\(^{2+}\) mobilization is not mediated by cAMP. Indeed PKA, by a mechanism that we have not addressed further, appears to cause desensitization of either muscarinic or PTH receptors.

Conclusions—We conclude, in keeping with previous work (29), that PTH-mediated Ca\(^{2+}\) mobilization does not result from production of IP\(_3\); PTH does not stimulate detectable formation of IP\(_3\), it has no detectable effect on the IP\(_3\) formation evoked by ATP or carbachol, and the lack of synergy between ATP and carbachol indicates that the IP\(_3\) formed in response to these receptors is incapable of mimicking PTH. Nor are the potentiated Ca\(^{2+}\) signals evoked by PTH mediated by cyclic AMP (Fig. 7), by Ca\(^{2+}\)-induced sensitization of IP\(_3\) receptors (Figs. 2, 4, and 5), or by phosphatidylinositol-3-kinase, because wortmannin (1 μM) did not prevent PTH from potentiating responses to carbachol (not shown). Our data are nevertheless consistent with IP\(_3\) receptors being involved in responses to PTH (Fig. 6). We suggest that activation of type 1 PTH receptors stimulates formation of an intracellular messenger that allows more complete emptying of intracellular Ca\(^{2+}\) stores than is possible with maximal concentrations of either ATP or carbachol alone. To account for the larger maximal response to carbachol in the presence of PTH (Fig. 3A), we suggest that PTH may facilitate translocation of Ca\(^{2+}\) between discrete intracellular Ca\(^{2+}\) stores (Fig. 8), such that stores lacking IP\(_3\) receptors are brought into continuity with those expressing them. Such a model would account for the small amount of Ca\(^{2+}\) release evoked by PTH alone (triggered by basal levels of IP\(_3\)) (Fig. 1C), for the increase in the size of the pool released by maximal concentrations of carbachol or ATP (Figs. 4 and 5B), and for the increased sensitivity of the stores to carbachol (Fig. 3A).

At present we can only speculate on the mechanisms linking PTH receptors to the transfer of Ca\(^{2+}\) between intracellular stores, although it is tempting to suggest that they may be related to the many reports linking small G proteins with Ca\(^{2+}\) translocation between intracellular stores. For example, previous studies had demonstrated that GTP facilitated transfer of Ca\(^{2+}\) between IP\(_3\)-sensitive and -insensitive stores by a process that required GTP hydrolysis and probably involved membrane fusion, the cytoskeleton, and a monomeric G protein (30–32).

Inositol 1,3,4,5-tetrakisphosphate has also been reported to between IP3-sensitive and -insensitive stores by a process, the cytoskeleton, and a monomeric G protein (30–32). Again monomeric G proteins are implicated, because the effects of inositol 1,3,4,5-tetrakisphosphate on IP\(_3\)-evoked Ca\(^{2+}\) mobilization appear to be mediated by a member of the GAP1 family of GTPase-activating proteins (33, 35). Indeed monomeric G proteins are involved in every aspect of intracellular membrane trafficking (36), including that between the organelles, endoplasmic reticulum and the Golgi, known to respond to IP\(_3\) (37).

Most extracellular stimuli cause Ca\(^{2+}\) mobilization by stimulating formation of the IP\(_3\) that gates intracellular Ca\(^{2+}\) channels. We suggest that PTH may be the first example of a hormone shown to regulate Ca\(^{2+}\) mobilization by regulating the size of the Ca\(^{2+}\) pool to which IP\(_3\) has access.

Acknowledgment—We thank Gavin Winston, who was supported by a grant from the Physiological Society, for help with experiments.

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