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Research progress in the stimulatory inputs regulating growth hormone (GH) secretion☆

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Abstract

A review is presented on progress in the research of stimulatory inputs that regulate growth hormone secretion, including recent results on the action of the hypothalamic peptides growth-hormone releasing factor (GHRH) and pituitary adenylate cyclase-activating polypeptide (PACAP), as well as that of both peptidic (growth hormone-releasing hexapeptide; GHRP-6) and non-peptidyl (L-163,255) synthetic GHSs on somatotrope cell function.

Keywords: Somatotropes; Growth hormone; Secretion; Second messengers; Signaling enzymes; Growth hormone-releasing factor (GHRH); Pituitary adenylate cyclase-activating polypeptide (PACAP); Synthetic growth hormone secretagogues

1. Introduction

It is becoming increasingly clear that the previously accepted model proposed to explain the hypothalamic regulation of growth hormone (GH) release from pituitary somatotropes, that is, the dual regulation by one stimulatory factor, growth-hormone releasing factor (GHRH), and one inhibitory, somatostatin (SRIF), is becoming obsolete and has to be expanded to include novel players (Frohman et al., 1992; Giustina and Veldhuis, 1998; Peter and Chang, 1999; McMahon et al., 2001). In particular, two classes of compounds are emerging as likely candidates to regulate GH secretion. Namely, pituitary adenylate cyclase-activating polypeptide (PACAP; Miyata et al., 1989; Arimura and Shioda, 1995; Vaudry et al., 2000) and the family of synthetic GH secretagogues (GHSs; Smith et al., 1996a,b; Bowers, 1999), of which a probable endogenous counterpart, a peptide termed ghrelin, has recently been identified, cloned and characterized (Kojima et al., 1999). There is also increasing evidence that in addition to these new extracellular regulators, the mechanisms that operate inside the somatotrope to interpret and respond to the instructions conveyed by such multiple regulatory inputs also include signaling pathways that were previously considered unimportant for this cell type (French et al., 1990; Bluet-Pajot et al., 1993). In our laboratory, we use the porcine somatotrope as a model to investigate the complexity of the control of GH secretion and

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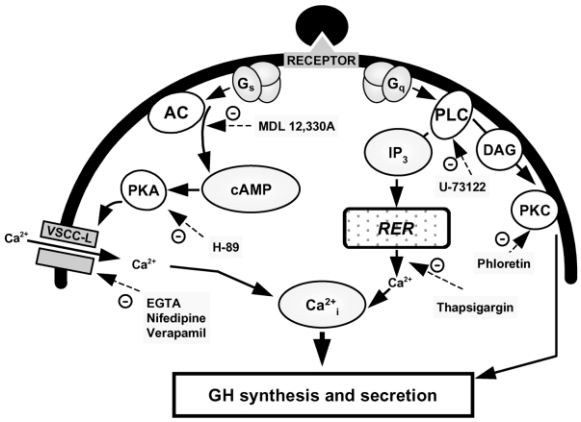

Fig. 1. Schematic diagram summarizing the main signaling pathways involved in the response of porcine somatotropes to regulatory factors and showing the points at which the different routes were blocked in our studies. *Abbreviations*: AC, adenylate cyclase; Ca_i^{2+}, cytosolic free calcium; DAG, diacylglycerol; Gs, Gq, heterotrimeric G proteins; IP_3, inositol 1,4,5-trisphosphate; VSCC-L, L-type voltage-sensitive Ca^{2+} channels; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; RER, rough endoplasmic reticulum.

the mechanisms underlying such control. Here, we review our recent results on the action of the hypothalamic peptides GHRH and PACAP, as well as that of both peptidic (growth hormone-releasing hexapeptide; GHRP-6) and non-peptidyl (L-163,255) synthetic GHSs on somatotrope cell function.

2. Major signaling pathways involved in somatotrope response to regulatory inputs

Functional responses of somatotropes to stimulatory factors can be mediated by the activation of several signaling systems, which can vary depending on the specific factor (Fig. 1). Classically, the adenylate cyclase (AC)/adenosine 3′,5′-cyclic monophosphate (cAMP)/protein kinase A (PKA) pathway has been considered as the main mechanism activated in this cell type in response to a number of stimuli, including GHRH (Frohman, 1996). On the other hand, the route of phospholipase C (PLC)/inositol phosphate (IP)/protein kinase C (PKC) was initially thought to play only a minor role in somatotrope function, yet recent studies have revealed this system as an important mediator in the secretory response of this cell type to certain secretagogues (Chen and Clarke, 1999).

The action of both the AC/cAMP/PKA system and the PLC/IP/PKC pathway on somatotrope cells, although activated through different intermediary GTP-binding proteins (presumably of the Gs and Gq type, respectively), ultimately results in net increases in the concentration of free cytosolic calcium ($[Ca^{2+}]_i$), which in turn lead to GH release (Chen et al., 1994; Herrington and Hille, 1994). In particular, PKA activation induces extracellular Ca^{2+} entry through voltage-sensitive Ca^{2+} channels (VSCC) (Takei et al., 1996), which in the case of somatotropes, are of the L- and T-type (Chen et al., 1990; Chen and Clarke, 1995). Furthermore, the activity of these channels is closely related to the function of other ion channels, such as K^+ and Na^+ channels, which are also regulated by a variety of factors acting on somatotrope cells (Kato and Suzuki, 1989; Ohlsson and Lindstrom, 1989; Kato and Sakuma, 1999). Regarding the IP/PLC/PKC system, both the inositol triphosphate (IP_3) and diacylglycerol (DAG) generated by PLC-mediated hydrolysis of phosphatidyl biphosphate (PIP_2) can contribute, through different mechanisms, to the $[Ca^{2+}]_i$ increases observed in somatotropes in response to different stimuli (Herrington and Hille, 1994; Bresson-Brépoldin and Dufy-Barbe, 1996; Smith et al., 1996a,b). Thus, IP_3 induces Ca^{2+} mobilization from the endoplasmic reticulum, whereas PKC, activated by DAG, can directly or indirectly stimulate extracellular Ca^{2+} influx. Finally, other signaling molecules, such as arachidonic acid and its metabolites, also seem to participate in the secretory response of somatotropes (Roudbaraki et al., 1996).

Attempts to elucidate the participation of specific transduction pathways in the response of somatotropes to a secretagogue can involve diverse experimental strategies. In our studies, two complementary approaches have been combined to delineate the intracellular mechanisms mediating the effects of PACAP, GHRH, GHRP-6 and L-163,255 on porcine somatotropes (Fig. 1). In the first approach, we assessed the direct effect of these substances on the levels of different second messengers, such as cAMP and IPs, in cultures of porcine pituitary cells, as well as the $[Ca^{2+}]_i$ in single immunoidentified somatotropes, after the administration of the corresponding secretagogue. In the second type of approach, we sought to ascertain the relative contribution of different intracellular signaling molecules to the secretory response of somatotropes. To this end, the effect of the secretagogues on GH release was evaluated

in cultures of dispersed adenohypophyseal cells after selectively blocking key enzymes, such as AC (by MDL 12,330A), PLC (by U73122), and PKA and PKC (by H-89 and phloretin, respectively), and also after blockade of extracellular Ca^{2+} influx (by EGTA or by the specific L-type VSCC blockers nifedipine or verapamil), or after depletion of intracellular Ca^{2+} stores (by thapsigargin).

3. Response of porcine somatotropes to PACAP

PACAP is a member of the secretin/glucagon/vasoactive intestinal peptide (VIP)/GHRH family (Arimura and Shioda, 1995; Rawlings and Hezareh, 1996; Vaudry et al., 2000) that exists in two bioactive molecular forms, one of 38 residues (PACAP38) and a shorter form of 27 amino acids (PACAP27), corresponding to the N-terminal part of PACAP38 (Miyata et al., 1989, 1990). Both forms of PACAP, which are present in the mammalian hypothalamus (Arimura and Shioda, 1995), stimulate GH release from somatotropes of several species (Goth et al., 1992; Hart et al., 1992; Hashizume et al., 1994; Sawangjaroen et al., 1997), thus supporting a role for PACAP as a hypophysiotropic factor (for review see Rawlings and Hezareh, 1996; Giustina and Veldhuis, 1998; Sherwood et al., 2000; Vaudry et al., 2000). In the case of the pig, we have recently demonstrated that both PACAP38 and PACAP27 specifically and directly increase GH mRNA levels and GH release in vitro (Martínez-Fuentes et al., 1998c). However, we found important differences in the dynamics of the response of somatotropes to each PACAP molecular form, both in terms of dose- and time-related parameters. Specifically, PACAP27-induced GH mRNA increase appeared faster (8 h) than that evoked by PACAP38 (16 h). In addition, the effect of the former on GH release was clearly dose-dependent, whereas that induced by the latter was not. Moreover, microfluorimetric studies carried out on single somatotropes revealed that both PACAPs increase $[Ca^{2+}]_i$ in a high percentage of cells, although PACAP38-induced $[Ca^{2+}]_i$ increases were higher than those produced by PACAP27 (Martínez-Fuentes et al., 1998b). It seemed likely, therefore, that the mechanisms mediating the effect of PACAP38 and PACAP27 on porcine somatotropes partially differed.

Such differences between PACAP27 and PACAP38 cannot be attributed to the AC/cAMP/PKA system, because inhibition of either of the two enzymes completely blocked GH release induced by both PACAPs (Martínez-Fuentes et al., 1998a). In addition, blockade of PKA significantly reduced the ability of the two peptides to increase $[Ca^{2+}]_i$ in single porcine somatotropes (Martínez-Fuentes et al., 1998b). Similarly, removal of extracellular Ca^{2+} and blockade of Ca^{2+} influx through L-type channels markedly diminished the response of somatotropes to PACAP38 and PACAP27, both in terms of Ca^{2+} responses and GH release (Martínez-Fuentes et al., 1998a,b).

In clear contrast, blockade of PLC reduced the stimulatory effect caused by PACAP38, but not that of PACAP27, indicating the participation of this enzyme in the action of PACAP38 on GH release by porcine somatotropes (Martínez-Fuentes et al., 1998a). Interestingly, the two signaling routes initiated by PLC, DAG-induced PKC activation and IP_3-dependent mobilization of Ca^{2+} from internal stores, seemed to differentially contribute to the effect of PACAP38. PKC depletion by long-term exposure to the phorbol ester TPA did not prevent PACAP38-induced GH release, whereas depletion of thapsigargin-sensitive endoplasmic reticulum Ca^{2+} stores partially reduced the effect of this peptide (Martínez-Fuentes et al., 1998a). In addition, and as would be expected, neither of these blockers modified the stimulatory action of PACAP27 on porcine GH release. In keeping with these findings, $[Ca^{2+}]_i$ response to PACAP38, but not to PACAP27, was partially diminished in conditions of PLC inhibition or after depletion of the endoplasmic reticulum-associated intracellular Ca^{2+} pool (Martínez-Fuentes et al., 1998b).

Identical results to those found for hormone release were observed for GH mRNA accumulation in somatotropes (Martínez-Fuentes et al., 1998a), thereby reinforcing our notion that PACAP38 and PACAP27 employ different molecular pathways to stimulate porcine somatotropes. The diagram shown in Fig. 2 outlines the major components of each pathway proposed. Both PACAP38 and PACAP27 stimulate GH synthesis and release in porcine somatotropes mainly by activating the AC/cAMP/PKA system, which in turn induces the required extracellular Ca^{2+} entry through L-type Ca^{2+}-channels. However, PLC activation and intracellular Ca^{2+} mobilization also partially contribute to PACAP38-, but not to PACAP27-induced stimulatory effects on porcine somatotropes. In summary, our results demonstrate

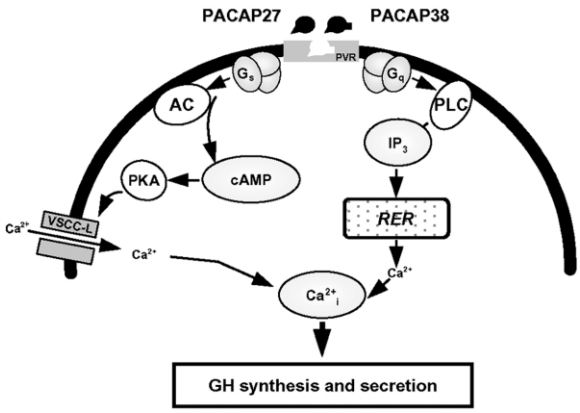

Fig. 2. Diagram showing the model proposed to explain the intracellular mechanisms employed by PACAP27 and PACAP38 to activate GH synthesis and secretion in porcine somatotropes. PACAP 27 would only activate the cAMP pathway shown in the left, whereas PACAP38 would require the activation of both the cAMP route and the PLC/IP_3 (right) cascade to exert its full action. PVR, PACAP/VIP receptor. See Fig. 1 for further abbreviations.

that these two molecular forms of PACAP act on porcine somatotropes by activating both common and distinct signaling mechanisms. Although the mechanisms underlying these differences are still unknown, it seems reasonable to propose that they likely relate to differential coupling of the PACAP receptor(s) expressed in porcine somatotropes to specific signaling pathways upon binding of each PACAP molecular form, as has been shown to occur in other cellular systems (Spengler et al., 1993; Pantaloni et al., 1996; Vaudry et al., 2000).

4. Response of porcine somatotropes to peptidic and non-peptidic GHSs

More than two decades ago, Cyril Bowers and his group synthesized a series of enkephalin-derived peptides that stimulated GH release in vitro, but lacked in vivo activity (Bowers et al., 1977). Bowers' group further modified these peptides based on conformational energy studies and structure-activity relationship 2 years before the isolation of the hypothalamic peptide GHRH, thus enabling the synthesis of a GH-releasing hexapeptide, termed GHRP-6, with a neat ability to specifically release GH in vivo and in vitro in a number of species, including rat, pig and human (Bowers et al., 1984, 1990; Doscher et al., 1984). The hexapeptide subsequently served as a model to design and synthesize a series of peptidic (GH-releasing peptides, GHRPs) and non-peptidic compounds that shared the ability of releasing GH, and were therefore termed growth hormone secretagogues or GHSs (Deghenghi, 1996; Smith et al., 1996a,b; Wyvratt, 1996). The availability of non-peptidic GHSs that, like L-163,255, possess high oral bioactivity and low toxicity (Gertz et al., 1993; Chang et al., 1996) enhanced the possible clinical significance of these compounds, thereby attracting more interest to this research field. Moreover, the discovery in 1996 of the receptor specific for GHSs and its abundance in the pituitary and hypothalamus in human, pig and rat (Howard et al., 1996; McKee et al., 1997; Yokote et al., 1998) strongly suggested the existence of a natural analogue of the synthetic GHSs. Indeed, in December 1999, a 28-amino-acid-residue peptide that appears to be the endogenous ligand for this heretofore orphan receptor was isolated, cloned and characterized from rat and human stomach and was named ghrelin (Kojima et al., 1999).

In our laboratory, we have employed the dual strategy mentioned earlier to investigate the in vitro response of porcine somatotropes to both peptidic (GHRP-6) and non-peptidyl (L-163,255) GHSs, as well as to elucidate the intracellular systems mediating these responses. Specifically, in the first set of studies, we found that GHRP-6 potently and rapidly stimulates porcine GH secretion in a dose-dependent manner. Furthermore, we observed that combined administration of GHRP-6 and GHRH induces an additive stimulation of GH release in vitro (Sánchez-Hormigo et al., 1998). These latter results contrast with the marked synergistic interaction found in vivo in pigs after co-administration of non-peptidyl GHSs and GHRH (Chang et al., 1993; Hickey et al., 1996), which can be observed even in animals previously subjected to hypophyseal stalk transection (Hickey et al., 1996), thus suggesting that the interaction between both compounds is directly exerted at the pituitary level. In view of these and our in vitro results, it is tempting to speculate that in the animal in vivo, there exists some type of factor or mechanism, of extrahypothalamic origin, that contributes to enhance the additive interaction observed in vitro between GHRH and GHS to a true synergistic stimulation of GH release.

The additive interaction effected by GHRH and GHRP-6 in our in vitro system also suggested that, as reported in other species (reviewed by Smith et al., 1996a,b), these peptides may act through

distinct signaling pathways within pig somatotropes. To examine this issue, we first measured phosphatidylinositol hydrolysis in porcine pituitary cells in response to GHRP-6 and/or GHRH. As previously reported for human and rat pituitary cells (Lei et al., 1995; Mau et al., 1995), GHRP-6 increased phosphatidylinositol turnover in porcine pituitary cells. In contrast, GHRH did not cause a significant rise in this activity, nor did it modify the effect caused by GHRP-6 (Sánchez-Hormigo et al., 1999). Consistent with these results, inhibition of PLC completely abolished GHRP-6-induced GH release and suppressed the additive effect evoked by the hexapeptide and GHRH, whereas it did not modify the stimulation caused by GHRH alone. Furthermore, similar results were obtained when activation of PKC, a subsequent step in the PLC signaling pathway, was prevented by phloretin, thus demonstrating that these enzymes play a requisite role for GHRP-6 to stimulate GH release from porcine somatotropes, and to additively interact with GHRH.

Having established the importance of the PLC/IP/PKC system in the response of porcine somatotropes to GHRP-6, we carried out a similar series of experiments to investigate the possible involvement of the AC/cAMP/PKA pathway in the response of this cell type to the hexapeptide and/or to GHRH. Contrary to results reported for other species (Cheng et al., 1989; Adams et al., 1996; Wu et al., 1996), we found that GHRP-6 significantly increased cAMP levels in porcine pituitary cells, yet to a minor extent than GHRH. Moreover, we observed that co-administration of GHRP-6 and GHRH caused an additive stimulation of cAMP production, thus suggesting that the increases evoked by each peptide are mediated through distinct mechanisms (Sánchez-Hormigo et al., 1999). Our results indicate the existence of dissimilarities among the mechanisms that can be activated by GHRP-6 in pituitary cells of different species, which may arise from the distinct coupling of the same receptor to different signaling pathways in each species. Nevertheless, the discovery of novel GHS receptors in the pituitary (Muccioli et al., 1998), coupled with the distinct actions of peptidic and non-peptidyl GHSs in somatotropes from pig (see below) and other species (Chen, 2000), would also support the notion that the response of this cell type to GHSs involves multiple receptors, which may differ depending upon the species and the GHS considered (Chen, 2000).

However, despite the ability of GHRP-6 to increase cAMP levels in porcine pituitary cells, we found that the activation of this pathway does not seem to be required for its stimulatory action on GH release. Indeed, blockade of AC or PKA did not affect GHRP-6-induced GH release, whereas in contrast, it abolished the stimulatory effect of GHRH (Sánchez-Hormigo et al., 1999). Furthermore, it is noteworthy that blockade of these enzymes inhibited the additive stimulation of GH release evoked by the combined administration of both peptides and in particular, inhibition of AC fully eliminated the GHRH/GHRP-6-induced GH release. Thus, although the activation of the cAMP pathway caused by GHRP-6 alone in porcine somatotropes is not necessary for the action of the hexapeptide on GH release, future studies should test its possible contribution to other cellular processes activated by GHRP-6, such as GH gene transcription (Castaño et al., 1998). On the other hand, our results underscore the crucial importance of the activation of the cAMP cascade for the interplay established between GHRH and GHRP-6 within porcine somatotropes to stimulate GH release. Consistent with this notion, in human (adenoma), rat or sheep pituitary cells, it has been shown that although GHRP-6 alone is unable to increase cAMP levels, it does enhance the GHRH-stimulated increase of cAMP (Cheng et al., 1989; Adams et al., 1996; Wu et al., 1996), thereby suggesting that in these species, this cascade would also play an important role in the additive interaction between both peptides.

Given the importance of the Ca^{2+} ion in the secretory response of somatotropes to diverse stimuli, we also tested the effect of GHRP-6 on $[Ca^{2+}]_i$ in single somatotropes by means of the indo-1 technique and the subsequent immunoidentification of measured cells. Using this methodology, we observed that the hexapeptide increased $[Ca^{2+}]_i$ in 83.3% of immunoidentified somatotropes by inducing three distinct patterns of $[Ca^{2+}]_i$ rise: a predominant Type I, or plateau-type response; a Type II, spike or transient response; and Type III, a biphasic or peak-plateau response (Sánchez-Hormigo et al., 1999). In rat somatotropes, where similar GHRP-6-induced $[Ca^{2+}]_i$ augmentation profiles have been reported, the predominant type of response corresponds to the type III or biphasic profile (Bresson-Brépoldin and Dufy-Barbe, 1994, 1996; Herrington and Hille, 1994). Furthermore, the use of specific inhibitors

has shown that extra- and intracellular Ca^{2+} selectively account for the different phases of such $[Ca^{2+}]_i$ rises. To be more specific, whereas the initial peak is due to the mobilization of Ca^{2+} from intracellular, thapsigargin-sensitive stores, and occurs in the absence of external Ca^{2+}, the second, plateau phase is sustained through the influx of extracellular Ca^{2+} via L-type VSCC (Bresson-Brépoldin and Dufy-Barbe, 1994). In pig somatotropes, we found that both the blockade of L-type VSCC by nifedipine and the depletion of intracellular Ca^{2+} pools by thapsigargin reduced the number of somatotropes responsive to GHRP-6, but did not cause a selective disappearance of the initial rise or the secondary phase in any of the response patterns. In fact, the three patterns of Ca^{2+} profiles were still observable, and retained an essentially identical morphology under such inhibitory conditions. Thus, in contrast with the mechanism observed in rat somatotropes, it seems that in pig somatotropes, both extracellular Ca^{2+} entry and Ca^{2+} mobilization from intracellular stores simultaneously contribute to the entire Ca^{2+} response induced by GHRP-6.

On the other hand, nifedipine and thapsigargin differentially affected the stimulatory action of GHRP-6 on GH secretion from pig somatotropes. Thus, the presence of nifedipine in the incubation medium clearly reduced the release of GH caused by GHRP-6 or GHRH alone, as well as by a combination of both peptides. Conversely, depletion of intracellular Ca^{2+} stores by thapsigargin did not affect the stimulation caused by the hexapeptide alone, whereas it decreased the effect of GHRH, as has been previously shown to occur in porcine somatotrope subpopulations (Ramírez et al., 1999), and it also substantially diminished the additive effect of both peptides. The effects of nifedipine on GHRP-6-induced GH release are in accordance with those found in rat somatotropes (Sartor et al., 1985; Bresson-Brépoldin and Dufy-Barbe, 1996) and with the reduction in the Ca^{2+}-response observed previously in single porcine somatotropes. On the other hand, the lack of an inhibitory effect of thapsigargin on GHRP-6-induced GH secretion contrasted with the data in rat (Bresson-Brépoldin and Dufy-Barbe, 1996) and with its effect on the Ca^{2+} response to the hexapeptide in single somatotropes. These latter results demonstrate again the existence of interspecies differences in the mechanism of action of GHRP-6. Moreover, they indicate that the mobilization of

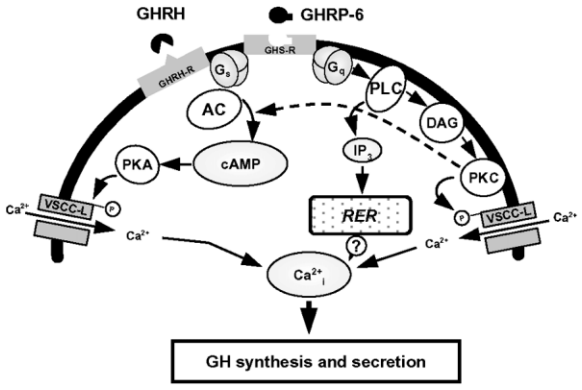

Fig. 3. Proposed working model of the possible signaling pathways and interactions underlying the response of porcine somatotropes to GHRH and GHRP-6. Dashed arrow indicates putative cross-talk between PKC and AC. See text for details. GHRH-R, receptor for GHRH; GHS-R, receptor for GHSs; other abbreviations as in Fig. 1.

Ca^{2+} from internal stores evoked by the hexapeptide in pig somatotropes is not required for its stimulatory effect on GH release when acting alone, although it seems to be important for its additive interaction with GHRH.

When taken together, the results obtained on the effects of GHRP-6 and GHRH in porcine somatotropes enable outline of a working model of the mechanism of action employed by these secretagogues to stimulate GH secretion in this cell type, which we have illustrated in the diagram in Fig. 3. Specifically, we propose that GHRP-6 stimulates GH release from porcine somatotropes through the sequential activation of the PLC/IP/PKC system, which in turn causes an extracellular Ca^{2+} influx through L-type VSCC. This mechanism of action clearly differs from that primarily employed by GHRH in this cell type, namely the AC/cAMP/PKA pathway. As a matter of fact, the ability of the hexapeptide to stimulate this latter pathway does not seem to be a requisite for its action on GH release when acting alone. On the other hand, most of the signaling molecules investigated herein, including cAMP, seem to be required for GHRP-6 and GHRH to additively stimulate GH release. In summary, our results suggest that the combined action of both peptides on porcine somatotropes involves a complex set of interlinked, cross-talking mechanisms, which likely converge at the cAMP level.

In addition to GHRP-6, the leading peptide model of GHSs, we have investigated the effects

and mechanisms of action of the non-peptidyl analogue developed by Merck, L-163,255, on porcine somatotropes (Chang et al., 1996). Similar to that found for the hexapeptide, L-163,255 potently and rapidly stimulated porcine GH secretion in a dose-dependent manner (Luque et al., 1998). Likewise, the analogue additively interacted with GHRH to stimulate GH release from cultured porcine somatotropes. This observation shows that the differences between the type of stimulatory interaction of GHRH and GHSs found in vivo (synergistic; Chang et al., 1993; Hickey et al., 1996) and in vitro (additive; Luque et al., 1998; Sánchez-Hormigo et al., 1999) are independent of the nature of the GHS considered (i.e. peptidic or non-peptidic). Furthermore, the use of the specific blockers U-73122 and phloretin also demonstrated that the action of the analogue was totally dependent on the activation of the PLC/IP/PKC transduction pathway, since inhibition of these enzymes severely reduced the GH release stimulated by the secretagogue, and also suppressed its additive interaction with GHRH. Interestingly, PLC activity not only appears to be important, but also essential for the interaction between GHRH and L-163,255, as its blockade fully abolished GH release above control levels.

In marked contrast with results previously found for GHRP-6, its non-peptidyl analogue did not stimulate cAMP production in cultures of porcine pituitary cells when applied alone (Luque et al., 1998). This finding reinforces our notion that multiple receptors could be involved in the porcine somatotrope response to distinct GHSs. Nevertheless, co-incubation of L-163,255 and GHRH potentiated the stimulatory action of the peptide on this parameter in a comparable manner to that found for other non-peptidyl GHSs (Cheng et al., 1993; Smith et al., 1993). Consistent with these findings, we observed that inhibition of AC did not modify the L-163,255-induced GH release, whereas in contrast, it reduced its additive stimulatory interaction with GHRH, thus confirming that the cAMP signaling pathway participates in the interplay established between GHSs and GHRH within porcine somatotropes.

Analysis of the Ca_i^{2+} dynamics in single cells demonstrated that L-163,255 increases $[Ca^{2+}]_i$ in somatotropes by inducing three types of profile, which are of similar morphology to those induced by GHRP-6, with the Type I or plateau being the predominant response profile. In spite of the similarities between the Ca^{2+} response induced by GHRP-6 and L-163,255, the contribution of extra- and intracellular Ca^{2+} sources to the GH release induced by the non-peptide analogue did not entirely correspond to that found for GHRP-6. Thus, both intracellular Ca^{2+} influx through L-type VSCC and mobilization of Ca^{2+} from internal stores contributed to the secretory response of porcine somatotropes to L-163,255, whereas, as mentioned earlier, the stimulatory effect of the hexapeptide on GH release is only partially dependent on extracellular Ca^{2+} and totally independent on Ca^{2+} from thapsigargin-sensitive intracellular stores. In summary, although the non-peptide analogue shares the main mechanisms employed by GHRP-6 to stimulate GH release in porcine somatotropes, namely the activation of the PLC/IP/PKC pathway, our present results indicate the existence of marked divergences between the signaling pathways underlying the effects induced by each secretagogue alone, as well as those activated when the synthetic compounds act in concert with GHRH. Such differences, which are summarized in the diagram shown in Fig. 4, include the relative importance of AC and PLC activity, and the contribution of the different Ca^{2+} sources in these responses.

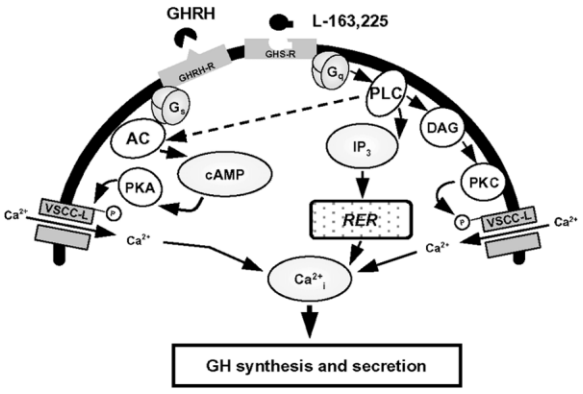

Fig. 4. Diagram outlining the working model proposed to explain the intracellular signaling pathways and possible interactions involved in the stimulatory response induced by the GHS L-163,255 and GHRH in porcine somatotropes. Dashed arrow indicates potential cross-talk between PLC and AC. See text for details. Abbreviations as in Figs. 1 and 3.

5. Concluding remarks

When viewed together, our results and those reported for other species (Chen and Clarke, 1999;

Chen, 2000) strongly support the increasingly accepted view that the diverse members of the GHS family act on somatotropes through multiple receptors and signaling mechanisms, which also markedly differ between species. Similarly, the response of somatotropes to the distinct forms of PACAP appears to be more complex than initially envisioned. This view, despite increasing the already complex picture of the intracellular mechanisms controlling GH release, will surely enhance our understanding on the functioning of this cell type. In conclusion, we believe that the GH axis is under a complex regulatory system that comprises multiple stimulatory and inhibitory factors, which by acting in the somatotrope through an intricate web of interlinked signaling cascades, ensure that GH secretion can be precisely regulated under diverse physiological circumstances.

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