peak increases over basal $^{4}\text{Ca}^\text{2+}$ accumulation of 78 ± 6% (100 nm-LHRH), 162 ± 14% (300 nm-TRH) and 235 ± 28% (60 nm-K$^+$) $(n = 6-12$, means ± s.e.m.). The response to K$^+$ was almost completely blocked by nimodipine with an IC$_{50}$ (concentration required to inhibit by 50%) of 4 ± 2 nm $(n = 7$, mean ± s.e.m.). A large proportion (approx. 70%) of the response to LHRH, but not TRH, was inhibited by similar concentrations of nimodipine. However, electrical recordings from gonadotrophs during application of LHRH show no voltage excursions sufficient to activate nimodipine-sensitive voltage-activated Ca$^2+$ channels (Mason et al., 1985). Therefore it is possible that, in the case of LHRH action, such channels can be activated by some means other than depolarization. In contrast, the response to TRH was not substantially inhibited by blockers of any of the known classes of voltage-activated Ca$^2+$ channels (nimodipine, 1 µM, phentoin, 100 µM; amiloride, 30 µM; Ni$^{2+}$, 100 µM; Cd$^{2+}$, 20 µM; ω-conotoxin, 1 µM), suggesting that it occurs through a distinct receptor-operated route.

The regulatory influence of protein kinase C on these Ca$^2+$ influx responses was assessed with phorbol esters and diacylglycerols and proved to be another means of distinguishing between the mechanisms involved. Whereas the response to 100 nm-LHRH was inhibited by 91 ± 10% in the presence of 100 nm-phorbol 12-myristate, 13-acetate; the response to 60 nm-K$^+$ was enhanced to 182 ± 16% and the response to 100 nm (or 2 nm)-TRH was completely unaffected $(n = 6-12$, means ± s.e.m.). Protein kinase C therefore appears to regulate neuropeptide-induced Ca$^2+$ influx only when the receptor-operated response involves nimodipine-sensitive Ca$^2+$ channels. Its action may therefore be at some relatively later stage in the receptor-induced signalling, perhaps at the nimodipine-sensitive Ca$^2+$ channel itself. Since voltage-activation of the channel is enhanced by phorbol esters, it is possible that protein kinase C may regulate the channel in two modes, differentially modifying its activation by voltage or receptor signals.

Abbreviations used: PKC, protein kinase C; PDBu, phorbol 12,13-dibutyrate; LH, luteinizing hormone; GH, growth hormone; H7, 1-(5-isoquinolinesulphonyl)-2-methylpiperazine dihydrochloride; NMD, nimodipine; IC$_{50}$ concentration required to inhibit by 50%.

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Phorbol ester-induced release of luteinizing hormone and growth hormone from rat anterior pituitary are differentially inhibited by the protein kinase C antagonists H7 and staurosporine.
These results, that there is a different temporal profile of release of LH and GH by PDBu, a differential action of two PKC inhibitors on this release and also that an L-type Ca\(^{2+}\) channel blocker can completely inhibit PDBu-induced release of GH but not LH, indicate strongly that regulation of hormone secretion by PKC is very differently organized in the cell types involved. It is known that LHRH-induced LH release is partly through Ca\(^{2+}\) -channels that can be blocked by dihydropyridines (Mitchell & Johnson, 1987; Chang et al., 1986). However, the release of LH by PDBu is obviously not through this route. In somatotrophes though, the activation of PKC would appear to cause GH secretion solely through these channels. Interestingly, a marked secretagogue action of Ba\(^{2+}\) is apparent on the release of GH but not LH (Mitchell & Anderson, 1985) consistent with voltage-activation of Ca\(^{2+}\) -channels occurring in the basal state of somatotrophes. Depolarization-induced \(^{45}\)Ca\(^{2+}\) influx through L-type Ca\(^{2+}\) -channels is also known to be enhanced by PKC (Fink et al., 1987) and this may be the mechanism underlying phorbol-induced GH secretion. The differential action of the two PKC inhibitors on PDBu-induced GH and LH secretion suggests that the PKC activated in gonadotrophes compared with somatotrophes may be either a different isoform or differently compartmentalized. There are presently seven subtypes of PKC identified (Nishizuka, 1988) which appear to be distinctly localized between tissues. In addition, these isoforms can be differentially activated, consequently it would not be unreasonable to assume that the present results reflect differential inhibition of PKC subtypes with H7 and staurosporine.

In conclusion, then, our data are consistent with either different isoforms, or modes of action, of PKC being involved in phorbol ester action on gonadotrophes and somatotrophes.

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Incorporation of arachidonic acid and its release from rat Leydig cells

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Previous work from this laboratory has shown that inhibition of the lipoxgenase (but not the cyclooxygenase) pathway of arachidonic acid metabolism inhibits luteinizing hormone (LH)-stimulated cyclic AMP and testosterone production in rat testis Leydig cells (Dix et al., 1984). These studies indicated that lipoxgenase products of arachidonic acid are involved in Leydig cell steroidogenesis. Arachidonic acid itself is stored in phospholipids; the nature of the latter and the mechanism by which arachidonic acid is released are, however, unknown in the Leydig cells. In this communication, we have investigated the kinetics and identity of the phospholipids into which \(^{14}\)C-arachidonic acid is incorporated and its subsequent release in the presence of LH.

Rat testis Leydig cells (98% purity and <1% macrophage contamination) were prepared by centrifugal elutriation followed by Percoll density gradient centrifugation (Platts et al., 1988). Analysis of phospholipids by h.p.l.c. (Shi-Hua Chen & Kou, 1982) showed that there are three main phospholipids present in the Leydig cell: these are phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylcholine (PC). Incubations with \(^{14}\)C-arachidonic acid showed that there was a rapid incorporation of the label into all three phospholipids within 2 h. Although the distribution of the total radioactivity incorporated into these phospholipids did not change, the incorporation of radioactivity continued to increase over 24 h. The distribution of the total radioactivity incorporated after incubation of the cells for 2 h was as follows: 19% (PI), 18% (PE) and 35% (PC).

Studies on the release of \(^{14}\)C-arachidonic acid from prelabelled Leydig cells in monolayer cultures, using h.p.l.c. to separate and quantify the arachidonic acid (Hirai et al., 1985), showed that LH causes a 2–3 fold increase in \(^{14}\)C-arachidonic acid released from the cells. This release was rapid in onset and reached a maximum 60 s after the addition of LH. Preliminary experiments also suggested that LH causes release of small amounts of arachidonic acid metabolites from Leydig cells. These were analysed by h.p.l.c. and one of these co-migrated with a standard 12-hydroxy eicosatetraenoic acid.

The release of \(^{14}\)C-arachidonic acid from prelabelled cells was also studied using a superfusion system. Leydig cells were pumped on to Teflon superfusion columns, containing Sephadex G-10 as an inert support, superfused with media maintained at 32°C at a flow rate of 1.0 ml/min, and the effluent collected as 1 min fractions. Superfusion of the cells with LH (for 2 min) caused a release of radioactivity which was rapid in onset and, reached a maximum within 1–2 min of LH addition.

These preliminary studies have demonstrated that \(^{14}\)C-arachidonic acid is incorporated into the PE, PI and PC of rat Leydig cells and is rapidly released upon addition of LH.

Abbreviations used: LH, luteinizing hormone; PI, phosphatidyl inositol; PE, phosphatidylethanolamine; PC, phosphatidylcholine.

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