Progesterone and oestradiol increase cytosolic Ca^{2+} in single rat hepatocytes

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Progesterone (300–400 μM) and oestradiol (25–200 μM) induce a prompt rise in the cytosolic free Ca^{2+} concentration (free Ca) in single rat hepatocytes, but testosterone, cortisol and dexamethasone do not. These increases are dependent on the presence of extracellular Ca^{2+} . Both progesterone and oestradiol block phenylephrine-induced free Ca oscillations. These data suggest a certain specificity of the response of free Ca to steroids and may explain some of the non-genomic effects of these steroids on hepatocytes.

INTRODUCTION

Classical concepts of the mechanism of steroid action at the nuclear level do not seem to be sufficient to account for all the known cellular effects of steroid hormones, including their rapid effects. Steroid hormones have a stimulatory effect on glycogen phosphorylase activity in liver [1–5] and in cultured rat hepatocytes [6], which precedes, and is independent of, protein synthesis. Studies from other laboratories have also shown some steroid-hormone effects which are independent of hormone interaction with the cell nucleus [7]. Binding sites in the hepatocyte plasma membrane for steroids have been found [8], and the presence of steroid-hormone receptors located in the plasma membrane has been proposed [9,10]. These reports suggest that the plasma membrane is a target for the action of steroids. Indeed, we have shown an interaction between steroids and liposome lipids [11].

It has been shown in liver [1–5] and cultured rat hepatocytes [6] that the rapid activation of glycogen phosphorylase by steroids does not involve an increase in cyclic AMP. The aim of this work was to investigate whether or not the steroid hormones increase free Ca in single hepatocytes, and whether a rise in free Ca could explain the activation of the hepatic glycogen phosphorylase mediated by these hormones.

MATERIALS AND METHODS

Hepatocytes were isolated from fed 180–250 g male Wistar-strain rats by perfusion of collagenase (Boehringer) as described previously [12]. Procedures for microinjection of single hepatocytes, the experimental conditions and data collection have been described previously [12]. Stock solutions of all the steroids (Sigma) were made daily in ethanol, except for cortisol, which was dissolved directly in the perfusion medium, Williams' Medium E (Flow Laboratories).

RESULTS

Progesterone (300 μM) induces a sustained rise in free Ca in a single rat hepatocyte (Fig. 1). The range of progesterone concentrations needed to induce an increase in free Ca was 300–400 μM (14 out of 17 hepatocytes responded), and the lag time ranged from seconds to 5 min. Fig. 2 shows the rises in free Ca induced by oestradiol, 25 μM (Fig. 2a) and 50 μM (Fig. 2b). The range of effective concentrations for oestradiol was 25–200 μM (40 out of 46 hepatocytes responded), and the lag time varied from 1 to 10 min. Either oestradiol induces a rise in free Ca that

Fig. 1. Effect of progesterone on free Ca levels in a rat hepatocyte

A single rat hepatocyte was microinjected with aequorin and superfused with 300 μM -progesterone (Prog) at the times indicated. The time constant for resting concentration of free Ca was 20 s, and for increases 2 s.

falls to resting levels even in the continued presence of the steroid (18 out of 40 hepatocytes; Fig. 2a), or free Ca does not fall to basal levels when the hormone is removed from the perfusate (Fig. 2b; 18 out of 40 hepatocytes). Only 4 out of 40 cells responded to oestradiol similarly to progesterone, with a rise that was sustained as long as steroid was present (results not shown). Testosterone (up to 300 μM), dexamethasone (up to 1 μM) and cortisol (up to 400 μM) did not increase free Ca (results not shown).

The influence of extracellular Ca^{2+} on free Ca increases induced by progesterone and oestradiol was studied. Fig. 3(a) shows that the rise in free Ca induced by progesterone (400 μM) is decreased

Abbreviation used: free Ca, cytosolic concentration of free Ca^{2+} .

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Fig. 2. Effect of oestradiol on free Ca levels

Two different single hepatocytes were superfused with (a) 25 μM - or (b) 50 μM -oestradiol (Oestradiol). The time constants and other details were as in Fig. 1.

to resting levels when the perfusate is replaced by Ca^{2+} -free medium containing 0.5 mM-EGTA (6 hepatocytes). Free Ca concentration takes approx. 5 min to return to resting levels. Free Ca oscillations induced by other agonists in single hepatocytes take a similar period of time to cease in Ca^{2+} -free medium with added EGTA [13]. Fig. 3(b) shows that, in the presence of EGTA-supplemented Ca^{2+} -free medium, oestradiol (25 μM) does not induce any change in free Ca. However, when Ca^{2+} is replaced in the perfusate a transitory increase in free Ca is induced (4 hepatocytes).

It has been shown that steroids have a synergistic action with Ca^{2+} -mobilizing agonists on hepatic glucose production [14], on glycogen phosphorylase activity in cultured hepatocytes [6] and

Fig. 3. Dependence of free Ca increases induced by progesterone and oestradiol on extracellular Ca^{2+}

Two different single hepatocytes were superfused in the presence or absence of Ca^{2+} -free medium (Ca^0) containing 0.5 mM-EGTA with (a) 400 μM -progesterone (Prog) or (b) 25 μM -oestradiol (Oestradiol). The time constants and other details were as in Fig. 1.

on phosphatidylinositol turnover [15]. However, Fig. 4 shows that progesterone (400 μM) irreversibly inhibits free Ca oscillations induced by phenylephrine (4 μM), an α_1 -adrenergic agonist (5 hepatocytes). The threshold concentration of progesterone for this inhibitory effect was 100–300 μM , depending on the hepatocyte. Oestradiol has a similar inhibitory effect on free Ca oscillations induced by phenylephrine (results not shown). The ethanol concentration in perfused medium never exceeded 1% (v/v); ethanol at this concentration did not induce free Ca changes.

DISCUSSION

The data shown here suggest a certain specificity in the free Ca response of hepatocytes to steroids, because only progesterone and oestradiol promote free Ca increases, whereas other steroids,

Fig. 4. Inhibitory effect of 400 μM -progesterone (Prog) on free Ca oscillations induced by 4 μM -phenylephrine (Phen)

The time constant for resting concentration of free Ca was 10 s, and for transients 1 s.

such as testosterone, cortisol and dexamethasone, do not. Similar increases in Ca^{2+} have been induced by progesterone in *Xenopus laevis* oocytes [9] and sperm [10,16], and by oestradiol in endometrial cells [17] and accessory glands [18]. The free Ca changes occur after a few minutes, even seconds, of perfusing the hepatocyte with the hormone, suggesting that steroid effects on the nucleus or on protein synthesis are not involved. Rapid effects of steroids described by several authors [6,7] were shown to be independent of protein synthesis, and had longer latency times than our free Ca responses.

Another feature that emerges from this study is the heterogeneity of free Ca responses when the hepatocytes are stimulated with oestradiol (Fig. 2), whereas progesterone gave always the same type of response (Fig. 1). Similar heterogeneity of free Ca responses to an agonist have been found in other cells [19] and recently in single hepatocytes stimulated with ATP [20]. The reasons for this heterogeneity are unclear. O'Sullivan *et al.* [19] have proposed that it is possible that the patterns of the cell responses may change in longer-term culture; however, this argument does not pertain, since the hepatocytes were used within a few hours after isolation.

It is generally accepted that a variety of agonists bind to their receptors, activating phosphoinositidase C through a guanine-nucleotide-binding protein. Phosphoinositidase C mediates the hydrolysis of plasmalemmal phosphatidylinositol 4,5-bisphosphate to generate *sn*-1,2-diacylglycerol, which is retained in the plasma membrane, and inositol 1,4,5-trisphosphate, which is responsible for the mobilization of intracellular Ca^{2+} , thereby causing a rapid increase in free Ca [21]. It has been shown that both progesterone and oestradiol stimulate phosphatidylinositol metabolism in other cell types [16,22]. However, Ca^{2+} release from intracellular reservoirs cannot be the only mechanism by which progesterone and oestradiol increase free Ca in single hepatocytes, because in the absence of extracellular Ca^{2+} the free Ca response does not happen (Fig. 3b). Usually, in hepatocytes, moderate concentrations of inositol 1,4,5-trisphosphate-mediated agonists induce oscillations of free Ca, although high

agonist concentrations can produce more sustained rises [23]. The dependence on external Ca^{2+} (Fig. 3) suggests that the mechanism by which these steroids raise free Ca is more likely to be due to an increase in Ca^{2+} influx across the plasmalemma, as has been shown in hepatocytes stimulated with other steroids [24], and in other cell types stimulated with progesterone or oestradiol [10,16,17]. A specific effect of progesterone and oestradiol directed at inhibition of the Ca^{2+} pump [25] is unlikely to be the sole effect on hepatocytes, since both steroids inhibit phenylephrine-induced spiking, even when free Ca is not elevated (Fig. 4).

Synergistic effects of steroid hormones with α_1 -agonists to activate glycogen phosphorylase in cultured hepatocytes have been shown [6]. However, both progesterone (Fig. 4) and oestradiol (results not shown) inhibit phenylephrine-induced spiking. It has been shown that oestradiol decreases [^3H]nor-adrenaline binding to synaptosomes [26], and progesterone diminishes the α -adrenoreceptor population in rabbit myometrial cells [27]. So, conceivably, these steroids could diminish the α -adrenoreceptor population through which phenylephrine induces Ca^{2+} mobilization. However, the fast effect of these steroids on phenylephrine-induced spiking argues against this possibility.

Although it has been reported that the high doses of steroid hormones required to provoke effects in the liver appear to be predominantly because of extensive metabolism of the steroids to inactive derivatives [28], the supraphysiological doses of steroid needed to induce effects on free Ca could also be the result of a non-specific effect of the steroids themselves on the plasma membrane, such as a change in fluidity [29]. However, such a non-specific action would be unlikely to be induced only by progesterone and oestradiol and not by steroids of closely similar chemical structure and molecular mass. A specific steroid-plasma-membrane interaction could be involved, as has been shown by using liposomes [11].

In conclusion, this work shows, for the first time, in hepatocytes that progesterone and oestradiol induce free Ca increases which could explain some of the non-genomic effects of these hormones.

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REFERENCES

- Egaña, M., Sancho, M. J. & Macarulla, J. M. (1981) *Horm. Metab. Res.* **13**, 609–611
- Díez, A., Sancho, M. J., Egaña, M., Trueba, M., Marino, A. & Macarulla, J. M. (1984) *Horm. Metab. Res.* **16**, 475–477
- Sanchez-Bueno, A., Sancho, M. J., Trueba, M. & Macarulla, J. M. (1987) *Int. J. Biochem.* **19**, 93–96
- Sancho, M. J., Egaña, M., Trueba, M., Marino, A. & Macarulla, J. M. (1986) *Exp. Clin. Endocrinol.* **88**, 249–255
- Sancho, M. J., Gomez-Muñoz, A., Sanchez-Bueno, A., Trueba, M. & Marino, A. (1988) *Exp. Clin. Endocrinol.* **92**, 154–160
- Gomez-Muñoz, A., Hales, P. J., Brindley, D. N. & Sancho, M. J. (1989) *Biochem. J.* **262**, 417–423
- Duval, D., Durant, S. & Homo-Delarche, F. (1983) *Biochim. Biophys. Acta* **737**, 409–442
- Trueba, M., Ibarrola, I., Ogiza, K., Marino, A. & Macarulla, J. M. (1991) *J. Membr. Biol.* **120**, 115–124
- Baulieu, E.-E., Schorderet-Slatkine, S., Le Gascogne, C. & Blondeau, J. P. (1985) *Dev. Growth Differ.* **27**, 223–231
- Blackmore, P. G., Beebe, S. J., Danforth, D. R. & Alexander, N. (1990) *J. Biol. Chem.* **265**, 1376–1380
- Sanchez-Bueno, A., Watanabe, S., Sancho, M. J. & Saito, T. (1991) *J. Steroid Biochem. Mol. Biol.* **38**, 173–179
- Sanchez-Bueno, A., Dixon, C. J., Woods, N. M., Cuthbertson, K. S. R. & Cobbold, P. H. (1990) *Biochem. J.* **268**, 627–632
- Woods, N. M., Dixon, C. J., Cuthbertson, K. S. R. & Cobbold, P. H. (1990) *Cell Calcium* **11**, 353–360

14. Exton, J. H., Friedmann, N., Wong, E. H. A., Brineaux, J. P., Cobbin, J. D. & Park, C. R. (1972) *J. Biol. Chem.* **247**, 3579–3588
15. Morishita, S. & Saito, T. (1989) *Jpn. J. Pharmacol.* **49**, 95–99
16. Thomas, P. & Meizel, S. (1989) *Biochem. J.* **264**, 539–546
17. Pietras, R. J. & Szego, C. M. (1975) *Nature (London)* **253**, 357–359
18. Batra, S. & Müntzing, J. (1981) *Eur. J. Pharmacol.* **76**, 87–91
19. O'Sullivan, A. J., Check, T. R., Moreton, R. B., Berridge, M. J. & Burgoyne, R. D. (1989) *EMBO J.* **8**, 401–411
20. Dixon, C. J., Woods, N. M., Cuthbertson, K. S. R. & Cobbold, P. H. (1990) *Biochem. J.* **269**, 499–502
21. Berridge, M. J. & Irvine, R. F. (1989) *Nature (London)* **341**, 197–205
22. Grove, R. I. & Korach, K. S. (1987) *Endocrinology (Baltimore)* **121**, 1083–1088
23. Cobbold, P. H., Sanchez-Bueno, A. & Dixon, C. J. (1991) *Cell Calcium* **12**, 87–96
24. Baran, D. T. & Milne, M. L. (1986) *J. Clin. Invest.* **77**, 1622–1626
25. Deliconstantinos, G. (1988) *Comp. Biochem. Physiol.* **89B**, 585–594
26. Inaba, M. & Kamata, K. (1979) *J. Steroid Biochem.* **11**, 1491–1497
27. Williams, L. T. & Lefkowitz, R. J. (1977) *J. Clin. Invest.* **60**, 815–818
28. Eisenfeld, A. J. & Aten, R. F. (1987) *J. Steroid Biochem.* **27**, 1109–1118
29. Rasmussen, H., Matsumoto, T., Fontaine, O. & Goodman, D. B. P. (1982) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41**, 72–77

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