The influence of plasma on basal and ACTH-stimulated *in vitro* adrenocortical steroidogenesis

P J Jenkins¹, T A Cross¹, L A Perry², S A Medbak², G M Besser¹
and A J L Clark¹

¹Department of Endocrinology, St Bartholomew's and The Royal London Hospital Medical School, Queen Mary and Westfield College, London, UK

²Immunoassay Laboratory, Department of Clinical Biochemistry, The Royal Hospitals' NHS Trust, London, UK

(Requests for offprints should be addressed to P J Jenkins, Department of Endocrinology, St Bartholomew's Hospital, West Smithfield, London EC1A 7BE, UK)

Abstract

Early descriptions of *in vitro* ACTH bioassays all emphasised the need to use extracted plasma samples due to interference by an unidentified component. The aim of these studies was to elucidate the effects of whole plasma on ACTH steroidogenic activity *in vitro* and to identify the responsible factor. A sensitive *in vitro* dispersed bovine adrenocortical cell bioassay was established. The addition of 10% ACTH-depleted human pooled plasma to the incubation media resulted in basal steroidogenesis equivalent to that achieved with 10^{-9} M ACTH₁₋₂₄ and potentiated the steroidogenic activity of 10^{-9} M ACTH₁₋₂₄ by 7·8-fold. This potentiation was dependent on the concentration of both ACTH and plasma in the media, but did not result from the mitogenic effect of plasma. A pituitary source was excluded and the potentiating activity was not extractable by Vycor glass. Column chromatography demonstrated two peaks of activity corresponding to molecular weights of 650 and 220×10^3 Da. These peaks did not correspond to the plasma binding of

¹²⁵I-ACTH which resulted from non-specific binding to albumin. Lipoprotein-deficient serum had no effect on either basal or ACTH-stimulated steroidogenesis, but both were restored by the addition of purified lipoproteins. However, novel findings demonstrated a differential effect of low (LDL) and high (HDL) density lipoproteins on basal and ACTH-stimulated steroid production; thus, LDL exerted a greater effect on the former, whilst HDL potentiated the steroidogenic activity of added ACTH more than LDL. The addition of the lipoproteins to lipoprotein-deficient serum restored its basal and ACTH potentiating effects, the cholesterol concentrations of the chromatographic fractions exactly paralleling their ACTH potentiating effect. These findings suggest that not only are lipoproteins the plasma factor(s) which potentiates ACTH steroidogenic activity in *in vitro* bioassays, but also that they exert differential effects on basal and ACTH-stimulated steroid production.

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Introduction

Prior to the introduction of its RIA, measurement of circulating adrenocorticotropin (ACTH) required the use of bioassays, although all of these emphasised the need for extracted plasma samples. In the case of *in vivo* assays, such as that developed by Lipscomb and Nelson (1962), this was to increase the assay sensitivity, whilst for *in vitro* bioassays, several workers observed that extraction was necessary to avoid interference in the assay by unidentified substances (Giordano & Sayers 1971, Lowry *et al.* 1973, Reader *et al.* 1982, Lambert *et al.* 1984). However, despite these observations, only a few preliminary attempts have been made to identify the responsible factor(s). Lowry *et al.* excluded prostaglandin E₂, which had been shown to have an effect in perfused adrenal glands, and insulin, which had been shown to increase the adrenal binding of radiolabelled corticotropin (Lowry *et al.* 1973, Rees *et al.*

1973). The modulatory effects of pro-opiomelanocortin (POMC) products other than ACTH, particularly with reference to the N-terminal region, have been investigated by a number of workers, but with conflicting results. Although N-POMC₁₋₇₆ has been shown to have a mild to moderate potentiating effect by some workers (Pedersen & Brownie 1980, Pedersen *et al.* 1980, Al-Dujaili *et al.* 1981), such actions have not been confirmed by other studies (Pham-Huu-Trung *et al.* 1982, Cathiard *et al.* 1985).

One possible explanation for any potentiation of *in vitro* ACTH bioactivity by whole plasma is that it might be due to a specific ACTH binding protein that protects the peptide from proteolytic degradation. This was supported by Upton *et al.* (1970) who performed crude chromatographic separation of plasma into high (>4·5 kDa) and low (<4·5 kDa) molecular weight fractions after infusion of human and porcine ACTH. They observed that 82% of

the bioactive human ACTH was in the high molecular weight fraction, compared with 31% of the porcine infusion plasma. Fehm *et al.* (1973) also suggested the presence of an ACTH binding protein and estimated its size to be $150\text{--}200 \times 10^3$ Da.

Thus, the existence of a substance in plasma that is capable of modulating the *in vitro* steroidogenic activity of ACTH has been documented by endocrinologists for over 20 years, but to date its identity has remained unknown. The aims of our investigation were: (1) to clarify the *in vitro* effect of human plasma on basal and ACTH-stimulated glucocorticoid synthesis by adrenal zona fasciculata and reticularis cells, (2) to characterise and identify any modulatory plasma factors, and (3) to clarify the existence of a specific plasma ACTH binding protein.

Materials and Methods

Dispersed adrenal cell bioassay

An *in vitro* dispersed adrenocortical ACTH bioassay was established based on previously described methodology (Tait *et al.* 1980, Bird *et al.* 1991). Fresh bovine adrenal glands were obtained from an abattoir within 20 min of slaughter. Each gland was slowly perfused, via the adrenal artery, with 30 ml warm Earles' balanced salt solution (EBSS) to remove red corpuscles, cut in a sagittal direction and the yellow medullary tissue removed. The underlying zona reticularis and fasciculata were gently scraped off leaving a 1-mm rim comprising the zona glomerulosa and capsule. The tissue from each gland was incubated for 90 min at 37 °C in 30 ml EBSS containing 2 mg/ml collagenase (Worthington Class 1; lot no. S4S088, Worthington Biochemical Corporation, Halls Mill Road, Freehold, NJ, USA). Any remaining clumps of tissue were further dispersed by vigorously bubbling air through the 50 ml tube using a Pipette-Aid; the resultant suspension was filtered over 250 µm nylon gauze and washed three times with warm EBSS. The number and viability of the dispersed cells was assessed using Trypan blue and a haemocytometer and cells were plated into 12-well plates at a concentration of 5×10^5 cells/well. One millilitre Ham's F12 medium, containing 10% fetal calf serum, penicillin 100 IU/ml, streptomycin 100 µg/ml and β-amphotericin 0.25 µg/ml was added to each well, and incubated for 24 h at 37 °C in 5% CO₂. Cells were then washed twice with warm EBSS containing 0.2% bovine serum albumin (BSA), after which they were ready for stimulation with the substances under investigation. In each experiment, the final volume in each well was made up to 500 µl with EBSS+0.2% BSA. Stimulated cells were incubated for a further 24 h, after which the incubation medium was carefully removed and stored at -20 °C prior to measuring the cortisol concentration by RIA. Using this protocol, the minimum detectable concentration of ACTH₁₋₂₄ was 5×10^{-12} M (34 pg/ml) with a

maximum response occurring between 10^{-9} and 5×10^{-9} M. Similar results were obtained with ACTH₁₋₃₉.

The intra- and interassay precisions for the steroidogenic effect of 10^{-9} M ACTH₁₋₂₄ were 8.3% and 67% respectively. Each study was performed in triplicate and each experiment repeated on three occasions. In order to overcome the interassay variability and to allow comparisons to be drawn between different experiments all values are expressed as a ratio of the steroidogenic effect of 10^{-9} M ACTH₁₋₂₄ which was used in each experiment.

Effect of human plasma on ACTH steroidogenic activity

Pooled plasma was used in which all the endogenous ACTH had been proteolytically cleaved by leaving at room temperature for 24 h, as evidenced by undetectable levels in an ACTH RIA. Ten percent by volume of plasma was added to the incubation media in each well, with and without ACTH₁₋₂₄ (final concentration 10^{-9} M). The effect of temperature was assessed by using plasma that had been heated to 37 °C or 55 °C for 12 h. Modulation by a POMC or pituitary factor was investigated by studying plasma samples from patients with (1) isolated ACTH deficiency, (2) panhypopituitarism following transphenoidal surgery and (3) following the administration of dexamethasone to normal subjects (0.5 mg 6-hourly for 48 h); the mean ACTH levels before and after dexamethasone were 23 pg/ml and <10 pg/ml respectively.

The mitogenic effect of plasma was assessed by measuring the total DNA content of the wells, according to the method of West *et al.* (1985).

Influence of Vycor glass extraction on ACTH potentiation

Three test samples (final volume 2 ml) were made up containing either 10% ACTH-deficient plasma, 10^{-8} M ACTH, or both combined. To each tube was added 1 ml silica glass 'Vycor' (previously activated by heating to 600 °C for 1 h) suspension (50 mg/ml water) and after gentle mixing for 30 min at room temperature, the supernatant was removed and stored at -20 °C. The remaining Vycor pellet was subjected to acid-acetone extraction with the extract being reconstituted in EBSS. Seventy-five microlitres of both the extracts and original supernatants were added to the assay wells with and without the addition of ACTH₁₋₂₄ (final concentration 10^{-9} M).

Estimation of the molecular weight of the modulatory factor

Chromatographic separation of plasma was performed using washed, degassed Sephacryl S300 (separation range of globular proteins of $10\text{--}1500 \times 10^3$ Da) in a 90 × 0.9 cm glass column. The eluant was EBSS+0.2% BSA at pH 7.4 passed at a rate of 6 ml/h with 1 ml fractions collected. The void volume (V_0) and total volume (V_t)

were determined using dextran blue and ^{125}I -ACTH respectively and the column calibrated with known molecular weight markers ($45\text{--}650 \times 10^3$ Da). After priming with plasma and equilibration for 48 h, 1 ml spun plasma was loaded onto the column. From each fraction 450 μl were added directly to the assay wells together with 50 μl 10^{-8} M ACTH; each fraction was assayed in duplicate.

Evaluation of an ACTH binding protein

Comparison was made of the radiochromatographic profiles after ^{125}I -ACTH had been incubated in plasma or EBSS and passed over the Sephacryl column. Both mixtures were incubated for 2 h at room temperature and eluted at a rate of 6 ml/h with 1 ml fractions collected. Subsequent experiments determined the effect of temperature (incubation at 4°C for 60 min) or mercaptoethanol upon the binding. The binding specificity was assessed by comparison with incubation in a physiological albumin solution (40 g/l) and the displacement by a 100-fold excess of 'cold' ACTH.

Specificity of plasma in potentiating ACTH-stimulated glucocorticoid synthesis

The specificity of the plasma potentiation to glucocorticoid secretion was assessed by the measurement of other adrenocortical hormones, androstenedione and testosterone, in the bioassay after stimulation by 10^{-9} M ACTH $_{1-24}$ and 10% plasma. Specificity to ACTH was assessed by observing the effects of plasma on glucocorticoid production in response to 10^{-8} and 10^{-9} M angiotensin II (AII).

Cholesterol as a modifier of ACTH steroidogenic activity

The influence of exogenous cholesterol upon the steroid production by the bioassay was assessed. Studies were performed using (1) lipoprotein-deficient serum (LPDS) and (2) purified high density (HDL) and low density (LDL) lipoproteins, prepared by density ultracentrifugation and membrane dialysis from the same plasma pool (Schumaker & Puppione 1986). The cholesterol concentrations of the purified LDL and HDL were 15.7 and 9.9 mmol/l respectively. To each bioassay well was added 50 μl LPDS or LDL or HDL without and with the simultaneous addition of 50 μl 10^{-8} M ACTH $_{1-24}$. Finally, comparison was made of the potentiating effect and the cholesterol content of the chromatographic fractions of plasma. Total cholesterol content was measured by an enzymatic assay (Nanjee *et al.* 1996).

Results

Effect of human plasma on ACTH steroidogenic activity

Although on its own 10% plasma resulted in steroidogenesis equivalent to 10^{-9} M ACTH $_{1-24}$ there was a marked

Figure 1 The effect of different proportions of plasma in the incubation medium on 10^{-9} M ACTH $_{1-24}$ -stimulated steroidogenesis. Values are the means $\pm$ S.E.M. of 3 experiments with each performed in triplicate.

synergistic effect when it was combined with 10^{-9} M ACTH $_{1-24}$, resulting in a 7.8-fold increase in cortisol production. There was no loss of basal or potentiating activity using plasma heated to either 37°C or 55°C . The potentiating effect was dependent on both the ACTH concentration and that of plasma, occurring with as little as 1% by volume. The addition of 20% and 50% plasma caused respectively a 1.6- and 3.2-fold increase in basal cortisol production compared with that from 10^{-9} M ACTH $_{1-24}$, and a 9.6- and 20-fold increase in 10^{-9} M ACTH $_{1-24}$ -stimulated steroid secretion (Fig. 1). The time course of the ACTH potentiation mirrored that of ACTH alone, both beginning within 30 min and reaching a maximum effect after 8 h incubation. A POMC or pituitary source of the potentiating factor was excluded because both the basal and ACTH potentiating activity of plasma from the three patient groups was the same as normal plasma.

That the potentiation was not a reflection of increased cell numbers was excluded by the DNA content of the wells. Although compared with 10^{-9} M ACTH 10% plasma on its own resulted in an increased DNA concentration, there was no further increase with the combination of plasma and ACTH.

Influence of Vycor glass extraction on ACTH potentiation

Figure 2 shows the potentiating effects of the extract and supernatant of plasma, ACTH or both combined on ACTH steroidogenic activity. As expected, the extract of the ACTH sample resulted in significant steroidogenesis, whilst the ACTH supernatant had minimal activity. In contrast, stimulation with the plasma extract resulted in little enhancement of steroidogenesis, whilst the supernatant resulted in a 3.1-fold potentiation.

Figure 2 The steroidogenic activity of the Vycor extract and supernatant of 10^{-9} M ACTH₁₋₂₄ ± plasma after addition to 10^{-9} M ACTH₁₋₂₄. Values are the means ± S.E.M. of 3 experiments with each performed in triplicate.

Figure 3 A representative graph of the enhancement of 10^{-9} M ACTH₁₋₂₄-stimulated steroidogenesis by chromatographic fractions obtained from plasma passed over a Sephacryl S300 column. Each value was performed in duplicate.

Estimation of the molecular weight of the potentiating factor

Figure 3 is a representative graph of the potentiating effect of fractions 25–92 on ACTH₁₋₂₄-stimulated steroidogenesis. Repeated experiments confirmed the findings. Two peaks of potentiation were obtained that corresponded to molecular weights of 650×10^3 and 220×10^3 Da respectively, but with the former being the more active.

Figure 4 The steroidogenic activity of LDL or HDL on basal steroidogenesis. Values are the means ± S.E.M. of 3 experiments with each performed in triplicate.

Evaluation of an ACTH binding protein

Column chromatography with radiolabelled ACTH showed that ^{125}I -ACTH₁₋₃₉ incubated with EBSS appeared after V_t , whilst after incubation with plasma there was a sharp peak that appeared at fraction 74, corresponding to an approximate molecular weight of 40×10^3 Da. This binding was unaffected by temperature or mercaptoethanol. An identical peak of binding was obtained following incubation in albumin and there was no displacement by the addition of excess 'cold' ACTH₁₋₃₉.

Specificity of plasma in potentiating ACTH-stimulated steroid synthesis

10^{-9} M ACTH stimulated the secretion of both androstenedione and testosterone into the medium, and secretion was potentiated 5.2- and 3.4-fold respectively by the addition of 10% plasma. Plasma also enhanced the production of cortisol in response to 10^{-8} and 10^{-9} M angiotensin-II by up to 18-fold.

The effects of cholesterol on ACTH steroidogenic activity

LPDS by itself caused no detectable steroidogenesis and no potentiation of ACTH. 10% LDL (final cholesterol concentration 1.57 mmol/l) resulted in basal steroidogenesis 0.6- and 0.47-fold respectively that obtained with 10% plasma and 10^{-9} M ACTH, whilst 10% HDL (final cholesterol concentration 1.0 mmol/l) was less potent, being 0.23- and 0.18-fold as effective respectively (Fig. 4). However, an opposite pattern of results was observed in their potentiation of 10^{-9} M ACTH₁₋₂₄ steroidogenic activity with 10% LDL causing a 4-fold increase (0.4 times as potent as whole plasma), whereas 10% HDL potentiated

Figure 5 The enhancement by LDL and HDL of 10^{-9} M ACTH₁₋₂₄-stimulated steroidogenic activity. Values are the means $\pm$ S.E.M. of 3 experiments with each performed in triplicate.

Figure 6 The cholesterol content and 10^{-9} M ACTH₁₋₂₄ enhancing activity of fractions obtained from chromatographic separation of plasma. Each value was performed in duplicate.

the effects of ACTH 9.2-fold (0.9 times as effective as whole plasma) (Fig. 5). In combination, their effects were additive with a 12.8-fold increase in steroid production. The cholesterol content of the chromatographic fractions paralleled their ACTH potentiating activity (Fig. 6).

Discussion

Although previous workers have suggested the existence of an ACTH binding protein (Fehm *et al.* 1972, Nicholson *et al.* 1984), our results suggest that such binding is due to non-specific interaction to albumin. Not only did the highest binding correspond to a molecular weight of approximately 40×10^3 Da, indistinguishable from that of albumin, but a similar peak was observed when 125 I-ACTH was incubated with a physiological concentration of albumin. Furthermore, plasma-bound 125 I-ACTH could not be displaced by the addition of excess 'cold' ACTH. These observations support those of Muramoto and Ramachandran (1981) who also documented binding of ACTH to albumin.

We have provided strong evidence that the factor(s) in human plasma that potentiates its *in vitro* steroidogenic activity is cholesterol in the form of lipoproteins. These findings explain the majority of earlier reports using *in vitro* dispersed adrenal cell bioassays, in which human plasma was observed to alter the shape of the steroidogenic dose-response curve (Giordano & Sayers 1971, Lowry *et al.* 1973, Lambert *et al.* 1984). The influence of lipoproteins on steroidogenesis has also been investigated by previous workers, although the majority of studies have been performed from a non-endocrinological perspective with the primary interest being directed towards the biochemistry of cholesterol *per se* and its subsequent uptake and metabolism, rather than the modulation of glucocorticoid secretion and synthesis. These studies on lipoprotein metabolism have revealed that LDL-cholesterol is preferentially utilised in mice (Faust *et al.* 1977) and human fetal cells (Simpson *et al.* 1979, Carr *et al.* 1980, Higashijima *et al.* 1987), whilst HDL-cholesterol is the preferred substrate in rats (Gwynne *et al.* 1976, Andersen & Dietschy 1978, Gwynne & Hess 1980). Studies using bovine adrenocortical cells have demonstrated variable results and few have directly compared the relative effects of HDL and LDL, although preincubation with either lipoprotein increased both basal and ACTH-stimulated steroidogenesis (Koper *et al.* 1985). No difference between the two was observed in ACTH-stimulated steroid production, even at high ACTH concentrations (10^{-7} M) (Higashijima *et al.* 1987), whilst another revealed HDL to have a greater effect on both basal and ACTH (10^{-7} M)-stimulated steroidogenesis (Yaguchi *et al.* 1998). Kovanen *et al.* (1979) observed that LDL but not HDL increased basal production, but did not investigate their influence on ACTH. A 5-fold increase in ACTH-stimulated (50 nM) cortisol production was recorded with human LDL after prolonged stimulation but comparison with human HDL was not made (Rainey *et al.* 1992). In addition, HDL, but not LDL, has also been reported to increase basal and AII-stimulated mineralocorticoid production by bovine zona glomerulosa cells (Knorr *et al.* 1988).

Our results from direct comparisons between LDL and HDL on basal and ACTH-stimulated steroid production are broadly in agreement with these studies (Koper *et al.* 1985, Knorr *et al.* 1988). We observed that both LDL and HDL increased basal secretion but with LDL being more potent, whilst opposite effects were observed on ACTH-stimulated steroidogenesis with HDL having a greater effect. The mechanisms that might explain these novel differential effects are uncertain. Under basal conditions, LDL might be preferentially taken up by adrenocortical cells, via endocytic internalisation of its specific receptor (Brown *et al.* 1979). The existence of such a receptor for HDL remains controversial and although attachment to the plasma membrane does occur, free cholesterol is preferentially taken up (up to 8-fold greater) rather than the intact molecule and its associated lipoprotein (Simpson 1979, Azhar *et al.* 1989, Reaven *et al.* 1989). Such uptake might be increased in the presence of ACTH.

An alternative explanation is that HDL is able to influence ACTH signal transduction. Alterations in membrane cholesterol:phospholipid ratio that influence its fluidity have been shown to affect membrane bound adenylate cyclase activity (Sinha *et al.* 1977, Sinensky *et al.* 1979, Whetton *et al.* 1983) and cause conformational transitions within the ACTH molecule that alter its interaction with the receptor (Nabedryk-Viala *et al.* 1978, Gysin & Schwyzer 1984). Functional studies have suggested that such alterations might have a physiological significance. Experimentally increasing the plasma membrane cholesterol:phospholipid ratio of adrenal cells, without adverse effects on cell viability, significantly increases production of ACTH-stimulated steroids and cAMP (Iida *et al.* 1987). Whether such membrane effects explain our results remains unproven. It is known that there is a bidirectional flux of cholesterol between cells and lipoproteins and the binding of HDL to the cell membrane can promote the translocation of cholesterol to the plasma membrane from intracellular stores (Johnson *et al.* 1986, Slotte *et al.* 1987). A combination of this and the preferential uptake of free cholesterol from HDL (Azhar *et al.* 1989, Reaven *et al.* 1989) might result in the cholesterol enrichment of adrenocortical plasma membranes leading to changes in membrane fluidity and thus ACTH binding and receptor conformation. Some circumstantial evidence in support of this is the observation that whilst HDL is able to potentiate ACTH-stimulated steroidogenesis, it does not suppress intracellular ACTH-promoted induction of HMG CoA reductase, unlike LDL (Rainey *et al.* 1992).

In conclusion, these observations suggest that lipoproteins are the principal constituents responsible for the interference by plasma of *in vitro* adrenocortical bioassays. In the absence of ACTH LDL is preferentially utilised for steroidogenesis, whilst in its presence HDL exerts a greater potentiating effect. The precise mechanisms for these differential effects remain to be determined.

References

- Al-Dujaili EAS, Hope J, Estivariz FE, Lowry PJ & Edwards CRW 1981 Circulating human pituitary pro- γ -melanotropin enhances the adrenal response to ACTH. *Nature* **291** 156–159.
- Andersen JM & Dietschy JM 1978 Relative importance of high and low density lipoproteins in the regulation of cholesterol synthesis in the adrenal gland, ovary, and testis of the rat. *Journal of Biological Chemistry* **253** 9024–9032.
- Azhar S, Stewart D & Reaven E 1989 Utilization of cholesterol-rich lipoproteins by perfused rat adrenals. *Journal of Lipid Research* **30** 1799–1810.
- Bird IM, Clyne CD, Lightly ERT, Williams BC & Walker SW 1991 Further characterisation of the steroidogenic responsiveness of purified zona fasciculata/reticularis cells from bovine adrenal cortex before and after primary culture: changing responsiveness to phosphoinositidase C agonists. *Journal of Endocrinology* **133** 21–28.
- Brown MS, Kovanen PT & Goldstein JL 1979 Receptor-mediated uptake of lipoprotein-cholesterol and its utilization for steroid synthesis in the adrenal cortex. *Recent Progress in Hormone Research* **35** 215–257.
- Carr BR, Porter JC, MacDonald PC & Simpson ER 1980 Metabolism of low density lipoprotein by human fetal adrenal tissue. *Endocrinology* **107** 1034–1040.
- Cathiard AM, Durand PH, Seidah MG, Chretien M & Saez JM 1985 Effects of several pro-opiomelanocortin-derived peptides on steroidogenesis in ovine and bovine adrenal cells. *Journal of Steroid Biochemistry and Molecular Biology* **23** 185–190.
- Faust JR, Goldstein JL & Brown MS 1977 Receptor-mediated uptake of low density lipoprotein and utilization of its cholesterol for steroid synthesis in cultured mouse adrenal cells. *Journal of Biological Chemistry* **252** 4861–4871.
- Fehm HL, Voigt KH & Pfeiffer EF 1972 Problems and artefacts in ACTH assay. *Hormone and Metabolic Research* **4** 477–481.
- Fehm HL, Voigt KH, Lang R, Ozyol MB & Pfeiffer EF 1973 Influence of plasma on ACTH-stimulated corticosterone production of isolated adrenal cells. *FEBS Letters* **36** 109–112.
- Giordano ND & Sayers G 1971 Isolated adrenal cells: assay of ACTH in rat serum. *Proceedings of the Society for Experimental Biology and Medicine* **136** 623–626.
- Gwynne JT & Hess B 1980 The role of high density lipoproteins in rat adrenal cholesterol metabolism and steroidogenesis. *Journal of Biological Chemistry* **255** 10875–10883.
- Gwynne JT, Mahaffee D, Brewer HB Jr & Ney RL 1976 Adrenal cholesterol uptake from plasma lipoproteins: regulation by corticotropin. *Proceedings for the National Academy of Sciences of the USA* **73** 4329–4333.
- Gysin B & Schwyzer R 1984 Hydrophobic and electrostatic interactions between adrenocorticotropin-(1–24)-tetracosapeptide and lipid vesicles. Amphiphilic primary structures. *Biochemistry* **23** 1811–1818.
- Higashijima M, Nawata H, Kato K & Ibayashi H 1987 Studies on lipoprotein and adrenal steroidogenesis. I. Roles of low density lipoprotein- and high density lipoprotein-cholesterol in steroid production in cultured human adrenocortical cells. *Endocrinologia Japonica* **34** 635–645.
- Iida S, Widmaier EP & Hall PF 1987 The influence of plasma membrane cholesterol on the response of adrenal cells to adrenocorticotropin. *Endocrinology* **120** 801–808.
- Johnson WJ, Bamberger MJ, Latta RA, Rapp PE, Phillips MC & Rothblat GH 1986 The bidirectional flux of cholesterol between cells and lipoproteins. Effects of phospholipid depletion of high density lipoprotein. *Journal of Biological Chemistry* **261** 5766–5776.
- Knorr M, Locher R, Vogt E, Vetter W, Block LH, Ferracin F, Lefkovits H & Pletscher A 1988 Rapid activation of human platelets by low concentrations of low-density lipoprotein via phosphatidylinositol cycle. *European Journal of Biochemistry* **172** 753–759.

- Koper WJ, Cordle SR & Yeaman SJ 1985 Effect of high and low density lipoproteins on corticotropin-mediated cortisol synthesis by bovine zona fasciculata cells. *Journal of Steroid Biochemistry and Molecular Biology* **23** 369–371.
- Kovanen PT, Faust JR, Brown MS & Goldstein JL 1979 Low density lipoprotein receptors in bovine adrenal cortex. I. Receptor-mediated uptake of low density lipoprotein and utilization of its cholesterol for steroid synthesis in cultured adrenocortical cells. *Endocrinology* **104** 599–609.
- Lambert A, Frost J & Robertson WR 1984 Preliminary experiences with a bioassay for adrenocorticotrophin (ACTH) in unextracted human plasma using dispersed guinea-pig adrenal cells. *Clinical Endocrinology* **21** 33–41.
- Lipscomb HS & Nelson DM 1962 A sensitive biological assay for ACTH. *Endocrinology* **71** 13–23.
- Lowry PJ, McMartin C & Peters J 1973 Properties of a simplified bioassay for adrenocorticotrophic activity using the steroidogenic response of isolated adrenal cells. *Journal of Endocrinology* **59** 43–55.
- Muramoto K & Ramachandran J 1981 Identification of the corticotropin binding domain of bovine serum albumin by photoaffinity labeling. *Biochemistry* **20** 3380–3385.
- Nabedryk-Viala E, Thiery C, Calvet P, Fermandjian S, Kisfaludy L & Thiery JM 1978 Conformation of corticotropin. An infrared spectrometry study of hydrogen exchange kinetics. *Biochimica et Biophysica Acta* **536** 252–262.
- Nanjee MN, Crouse JR, King JM, Hovorka R, Rees SE, Carson ER, Morgenthaler J, Lerch P & Miller NE 1996 Effects of intravenous infusion of lipid-free apo A-I in humans. *Arteriosclerosis and Thrombosis and Vascular Biology* **16** 1203–1214.
- Nicholson WE, Davis DR, Sherrell BJ & Orth DN 1984 Rapid radioimmunoassay for corticotropin in unextracted human plasma. *Clinical Chemistry* **30** 259–265.
- Pedersen RC & Brownie AC 1980 Adrenocortical response to corticotropin is potentiated by part of the amino-terminal region of pro-corticotropin/endorphin. *Proceedings of the National Academy of Sciences of the USA* **77** 2239–2243.
- Pedersen RC, Brownie AC & Ling N 1980 Pro-adrenocorticotropin/endorphin-derived peptides: coordinate action on adrenal steroidogenesis. *Science* **208** 1044–1046.
- Pham-Huu-Trung MT, de Smutter N, Bogio A, Bertagna X & Girard F 1982 Responses of isolated guinea-pig adrenal cells to ACTH and pro-opiocortin-derived peptides. *Endocrinology* **110** 1819–1821.
- Rainey WE, Rodgers RJ & Mason JI 1992 The role of bovine lipoproteins in the regulation of steroidogenesis and HMG-CoA reductase in bovine adrenocortical cells. *Steroids* **57** 167–173.
- Reader SCJ, Daly JR & Robertson WR 1982 Effect of human plasma on the bioactivity of adrenocorticotrophin. *Journal of Endocrinology* **92** 449–455.
- Reaven E, Spicher M & Azhar S 1989 Microvillar channels: a unique plasma membrane compartment for concentrating lipoproteins on the surface of rat adrenal cortical cells. *Journal of Lipid Research* **30** 1551–1560.
- Rees LH, Ratcliffe JG, Besser GM, Kramer R, Landon J & Chayen J 1973 Comparison of the redox assay for ACTH with previous assays. *Nature New Biology* **241** 84–85.
- Schumaker VN & Puppione DL 1986 Sequential flotation ultracentrifugation. *Methods in Enzymology* **128** 155–170.
- Simpson ER 1979 Cholesterol side-chain cleavage, cytochrome P450, and the control of steroidogenesis. *Molecular and Cellular Endocrinology* **13** 213–227.
- Simpson ER, Carr BR, Parker CR Jr, Milewich L, Porter JC & MacDonald PC 1979 The role of serum lipoproteins in steroidogenesis by the human fetal adrenal cortex. *Journal of Clinical Endocrinology and Metabolism* **49** 146–148.
- Sinensky M, Minneman KP & Molinoff PB 1979 Increased membrane acyl chain ordering activates adenylate cyclase. *Journal of Biological Chemistry* **254** 9135–9141.
- Sinha AK, Shattil SJ & Colman RW 1977 Cyclic AMP metabolism in cholesterol-rich platelets. *Journal of Biological Chemistry* **252** 3310–3314.
- Slotte JP, Oram JF & Bierman EL 1987 Binding of high density lipoproteins to cell receptors promotes translocation of cholesterol from intracellular membranes to the cell surface. *Journal of Biological Chemistry* **262** 12904–12907.
- Tait JF, Tait SAS & Bell JBG 1980 Steroid hormone production by mammalian adrenocortical dispersed cells. *Essays in Biochemistry* **16** 99–174.
- Upton GV, Hollingsworth DR, Londe S, Lerner AB & Amatrudda TT Jr 1970 Comparison of purified and porcine ACTH in man. *Journal of Clinical Endocrinology and Metabolism* **30** 190–195.
- West DC, Sattar A & Kumar S 1985 A simplified *in situ* solubilization procedure for the determination of DNA and cell number in tissue cultured mammalian cells. *Analytical Biochemistry* **147** 289–295.
- Whetton AD, Gordon LM & Houslay MD 1983 Elevated membrane cholesterol concentrations inhibit glucagon-stimulated adenylate cyclase. *Biochemical Journal* **210** 437–449.
- Yaguchi H, Tsutsumi K, Shimono K, Omura M, Sasano H & Nishikawa T 1998 Involvement of high density lipoprotein as substrate cholesterol for steroidogenesis by bovine adrenal fasciculo-reticularis cells. *Life Sciences* **62** 1387–1395.

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