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THE RELATIONSHIP BETWEEN ADRENAL VASCULAR EVENTS AND STEROID SECRETION: THE ROLE OF MAST CELLS AND ENDOTHELIN

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Summary—The actions of ACTH on the adrenal cortex are known to be 2-fold. In addition to increased steroidogenesis, ACTH also causes marked vasodilation, reflected by an increased rate of blood flow through the gland. Our studies, using the *in situ* isolated perfused rat adrenal preparation, have shown that zona fasciculata function and corticosterone secretion are closely related to vascular events, with an increase in perfusion medium flow rate causing an increase in corticosterone secretion, in the absence of any known stimulant. These observations give rise to two important questions: how does ACTH stimulate blood flow; and how does increased blood (or perfusion medium) flow stimulate steroidogenesis? Addressing the first question, we have recently identified mast cells in the adrenal capsule, and shown that Compound 48/80, a mast cell degranulator, mimics the actions of ACTH on adrenal blood flow and corticosterone secretion. We have also demonstrated an inhibition of the adrenal vascular response to ACTH in the presence of disodium cromoglycate, which prevents mast cell degranulation. We conclude, therefore, that ACTH stimulates adrenal blood flow by its actions on mast cells in the adrenal capsule. Addressing the second question, we looked at the role of endothelin in the rat adrenal cortex. Endothelin 1, 2 and 3 caused significant stimulation of steroid secretion by collagenase dispersed cells from both the zona glomerulosa and the zona fasciculata. A sensitive response was seen, with significant stimulation at an endothelin concentration of 10^{-13} mol/l or lower. Endothelin secretion by the *in situ* isolated perfused rat adrenal gland was measured using the Amersham assay kit. Administration of ACTH (300 fmol) caused an increase in the rate of immunoreactive endothelin secretion, from an average of 28.7 ± 2.6 to 52.6 ± 6 fmol/10 min ($P < 0.01$, $n = 5$). An increase in immunoreactive endothelin secretion was also seen in response to histamine, an adrenal vasodilator, which stimulates corticosterone secretion in the intact gland, but has no effect on collagenase-dispersed cells. From these data we conclude that endothelin may mediate the effects of vasodilation on corticosterone secretion, and this mechanism may explain some of the differences in response characteristics between the intact gland and dispersed cells.

INTRODUCTION

The mammalian adrenal gland is a highly vascular organ. In the rat, although the adrenals comprise only about 0.02% of the total body weight, they receive around 0.14% of the cardiac output [1]. The adrenal vasculature is arranged such that every adrenocortical cell is adjacent to a blood vessel [2]. Corticotrophin (ACTH) is an important regulator of adrenocortical function, and several studies have shown that, in addition to having a direct effect on steroidogenesisis, ACTH also has an effect on the adrenal vasculature, and acts to increase the rate of blood (or perfusion medium) flow through the adrenal gland [3–8]. It appears likely that ACTH may cause dilation of capsular and subcapsular arterioles [9].

The isolated perfused *in situ* rat adrenal preparation is well-suited to the study of the adrenal vasculature and its relationship to adrenal function. In this preparation, unlike conventional *in vitro* adrenocortical preparations, the architecture of the adrenal remains intact, and the gland is supplied with perfusion medium through the normal arterial route [10, 11].

Using the perfused adrenal preparation, previous studies from this laboratory have demonstrated a close relationship between the rate of perfusion medium flow through the adrenal gland

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and the rate of corticosterone secretion, such that an increase in the rate of delivery of perfusion medium to the gland causes an increase in the rate of corticosterone secretion, even in the absence of trophic hormone stimulation [12]. This suggests a specific relationship between vascular events and inner zone function, since corticosterone is a specific marker of inner zone (zonae fasciculata/reti-cularis) function in this preparation, and does not appear to be secreted by the zona glomerulosa [11].

These observations raise two important questions: firstly, how does ACTH cause vasodilation in the adrenal gland; and secondly, how do vascular changes influence steroidogenesis?

In an attempt to answer these questions, the present studies have looked at the function of mast cells located in the adrenal capsule, and the effects on steroidogenesis of some of the secretory products of the vascular endothelium.

MATERIALS AND METHODS

Animals used were Wistar rats weighing 250–350 g, bred and maintained in the animal house of St Bartholomew's Hospital Medical College. In the perfusion experiments, only male animals were used, while both male and female animals were used for the cell experiments.

Adrenal perfusion experiments

The method for perfusing the left adrenal of the rat was as described previously [10, 11]. Briefly, medium was introduced, via a cannula in the coeliac artery, into an isolated segment of aorta from which the adrenal arteries arise, and collected from a pocket in the renal vein. The gland was totally isolated from the systemic circulation, and received only perfusion medium. The perfusion medium used in these studies was tissue culture medium 199 (Difco Laboratories, Detroit, MI, U.S.A.) modified by dilution (4:3) with KCl-free Krebs Ringer bicarbonate, to give a final potassium ion concentration of 3.9 mmol/l, with bovine serum albumin (5 g/l; fraction V, Sigma, Poole, Dorset, England). Perfusion medium was delivered at a constant rate of about 0.8 ml/min throughout these experiments. After an initial equilibration period of 40 min, consecutive 10-min samples were collected.

While the gland received only perfusion medium, not all the medium supplied passes through the gland, some being lost through the fine arterioles arising from the adrenal arteries. Under basal conditions about 30% of the medium supplied reaches the gland, but this varies with different conditions of stimulation which cause changes in the vascular resistance of the gland. The rate of perfusate flow was monitored by measuring the volume of each 10-min sample collected, before the steroids were extracted from the sample with ethyl acetate. Aldosterone and corticosterone were measured by RIA [13, 14].

In the experiments where endothelin-immunoreactivity was measured, perfusate samples were collected on ice, and a 300 μl aliquot of each sample was immediately frozen and stored at $-20°C$ for endothelin assay. Endothelin-immunoreactivity was measured using the endothelin-1 assay kit supplied by Amersham U.K. (Bucks., England). This assay measures immunoreactive endothelin 1 and 2 (cross reactivities 100 and 204%, respectively), and the sensitivity of the assay is 1.3 fmol/tube (data supplied by Amersham).

Experimental protocols

All stimulants were dissolved in perfusion medium to the required concentration, and delivered as a bolus infusion (200 μl) directly into the coeliac cannula at the start of a 10-min collection period. Histamine, Compound 48/80 and sodium cromoglycate (cromolyn, a mast cell stabilizer) were obtained from Sigma, and ACTH (1–24) (Synacthen) from Ciba-Geigy (Horsham, Sussex, England). In the experiments with sodium cromoglycate, this compound was administered 60 s before the administration of ACTH.

Cell experiments

Adrenal glands were cleaned of adhering fat, and decapsulated between glass plates to separate the zona glomerulosa tissue from the inner zones (zonae fasciculata/reticularis). The inner zones were incubated in Krebs Ringer bicarbonate containing 2 g/l bovine serum albumin, and 2 g/l glucose (KRBGA), con-taining 2 mg/ml collagenase (Worthington type 1: Lorne diagnostics, Bury St Edmonds, Suffolk, England) for 1 h at 37°C. Cells were dis-aggregated by repeated pipetting, then pelleted by centrifugation at 500 *g* for 10 min. The supernatant was discarded and the cells were washed in fresh

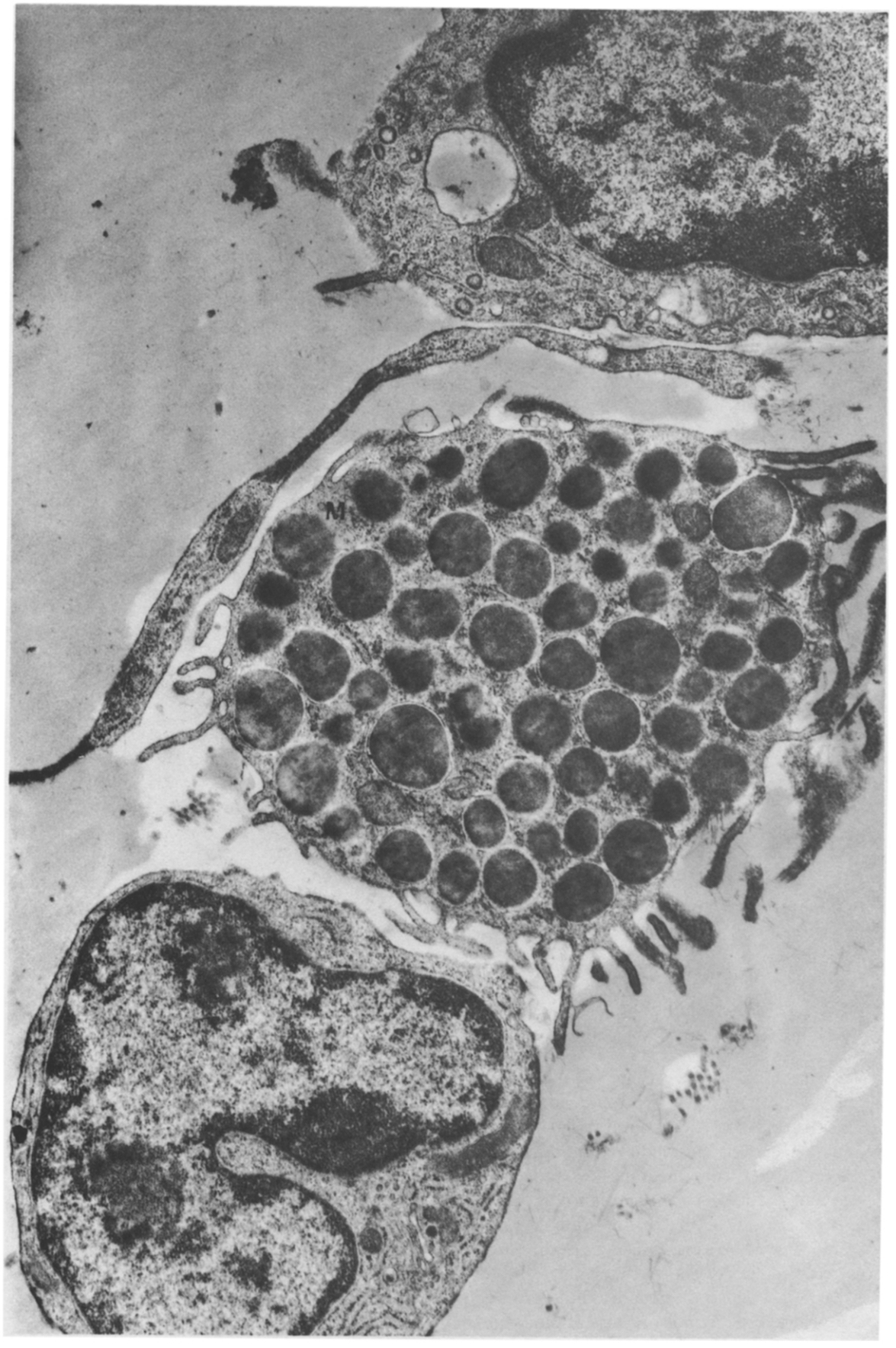

Fig. 1. Mast cell (M) in the adrenal capsular region, ×9000. Micrograph courtesy of Professors M. M. and M. C. Magalhaes and Dr D. Pignatelli.

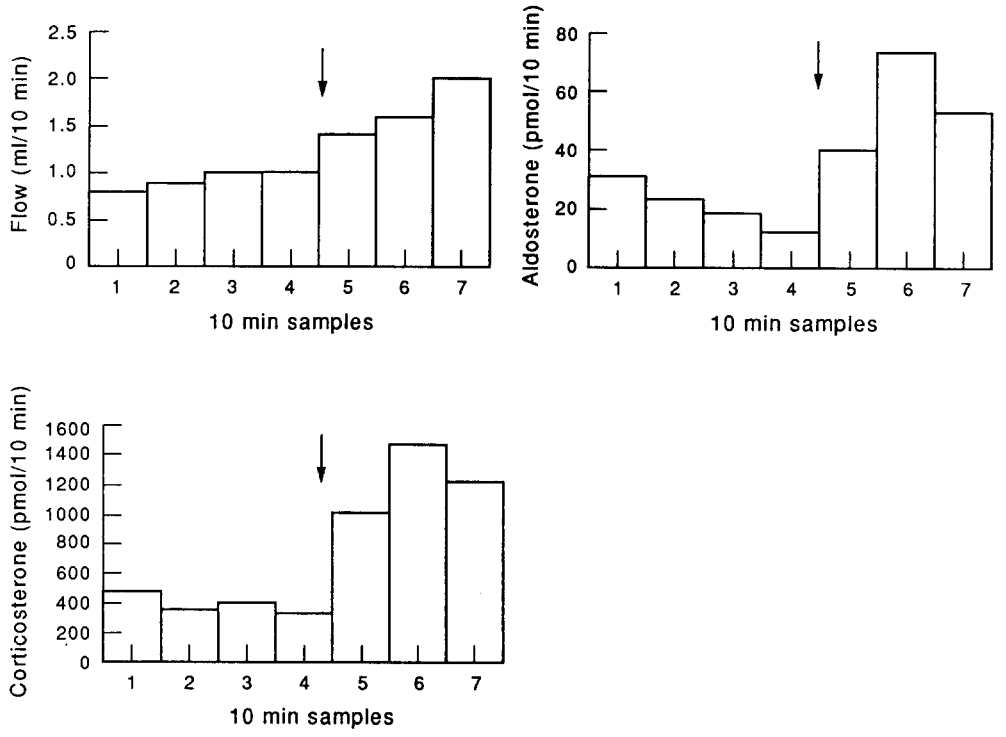

Fig. 2. An example of a perfusion experiment, showing the effects of 0.5 mg Compound 48/80 (time of administration indicated by the arrow) on adrenal perfusate flow rate and aldosterone and corticosterone secretion. In a series of experiments, this dose of Compound 48/80 caused a mean increase in flow rate of $90 \pm 11\%$ ($P < 0.001$), an increase in aldosterone secretion of $340 \pm 82\%$ ($P < 0.01$) and an increase in corticosterone secretion of $260 \pm 45\%$ ($P < 0.01$); $n = 6$ in all cases. Data taken from Ref. [23].

KRBGA, and recentrifuged. The cell pellet was resuspended in fresh KRBGA and incubated (approx. 1×10^5 cells per flask, final incubation vol 5 ml) for 2 h at 37°C in the presence or absence of endothelin 1, 2 or 3 (from Bachem U.K.; Saffron Waldon, Essex, England). Incubation media were extracted and assayed as described above.

RESULTS

Mast cells

Figure 1 shows the presence of a mast cell in the capsular region of the rat adrenal gland. The administration of Compound 48/80 to the isolated perfused rat adrenal caused an increase in the rate of perfusion medium flow and aldosterone and corticosterone secretion (Fig. 2). This agent had no effect on the rate of steroid secretion by collegenase-dispersed adrenocortical cells (data not shown).

The mast cell stabilizer, disodium cromoglycate caused a significant attenuation of the response to ACTH: the flow rate increment was virtually abolished, and the corticosterone response was reduced by about 50%. The aldosterone response to ACTH was unaffected by disodium cromoglycate (Table 1).

Endothelin

Endothelin 1, 2 and 3 each caused a significant stimulation of corticosterone secretion by collagenase-dispersed zona fasciculata/reticularis cells. The minimum concentration of peptide which caused significant stimulation of corticosterone secretion was 10^{-13} mol/l endothelin 1 and 10^{-14} mol/l endothelin 2 and 3 (Fig. 3).

Table 1. Effects of the mast cell stabilizer, disodium cromoglycate (DSCG; 10 μg), administered 60 s before administration of ACTH (3 pmol) on the perfusate flow rate and aldosterone and corticosterone response to ACTH in the isolated perfused rat adrenal gland *in situ*

	ACTH (3 pmol)	DSCG + ACTH (3 pmol)
Flow (% increase)	86 ± 10	16 ± 11***
Aldosterone (% increase)	288 ± 29	269 ± 24
Corticosterone (% increase)	402 ± 78	216 ± 36*

Values are means ± SEM of 9 experiments. *$P < 0.05$, ***$P < 0.001$, compared with the effect of ACTH alone (Student's t-test).

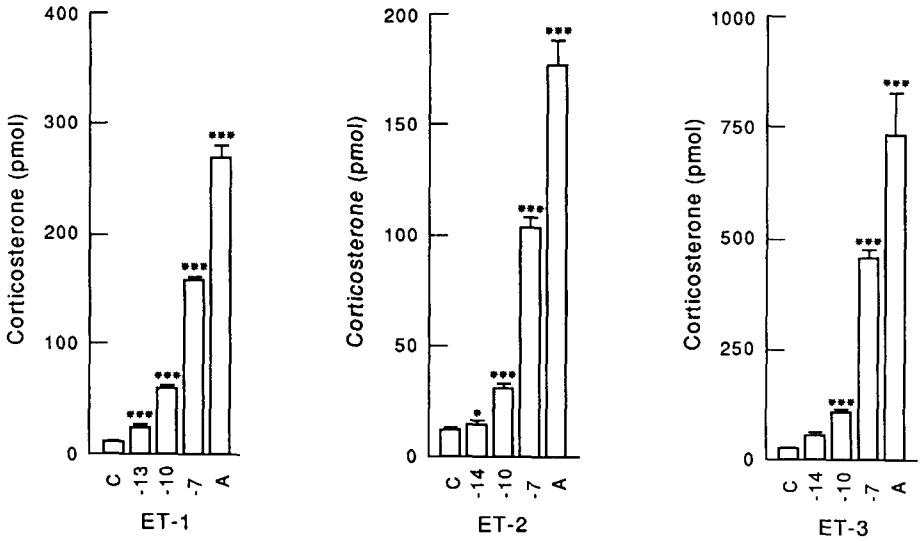

Fig. 3. Actions of endothelin 1, 2 and 3 on corticosterone secretion by rat adrenal zona fasciculata cells *in vitro*. C, Control; A, ACTH (10^{-7} mol/l); numbers on X-axis refer to the log concentration of endothelin used. $n = 4$ in each case. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (Student's t-test).

ACTH and histamine both caused an increase in perfusion medium flow rate, corticosterone and aldosterone secretion, and additionally both these agents provoked an increase in the output of immunoreactive endothelin from the isolated perfused rat adrenal gland (Figs 4 and 5).

DISCUSSION

From the results presented here, it is now possible to present an overall hypothesis to explain the link between blood flow and steroid hormone secretion in the rat adrenal cortex.

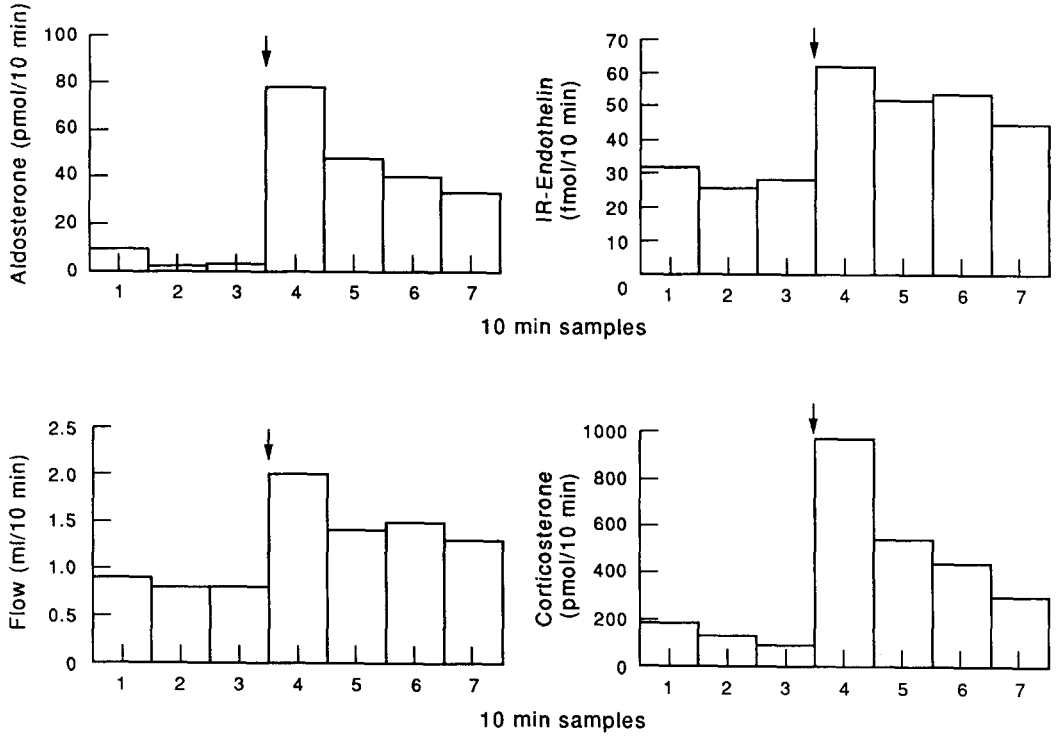

Fig. 4. An example of an experiment showing the effects of 300 fmol ACTH (time of administration indicated by the arrow) on: flow rate and corticosterone, aldosterone and endothelin secretion by the intact perfused rat adrenal. In a series of 5 experiments this dose of ACTH caused an average 85 ± 7% increase in flow rate ($P < 0.001$), a 370 ± 100% increase in aldosterone secretion ($P < 0.01$), a 490 ± 130% increase in corticosterone secretion ($P < 0.01$) and a 95 ± 10% increase in immunoreactive endothelin secretion ($P < 0.001$).

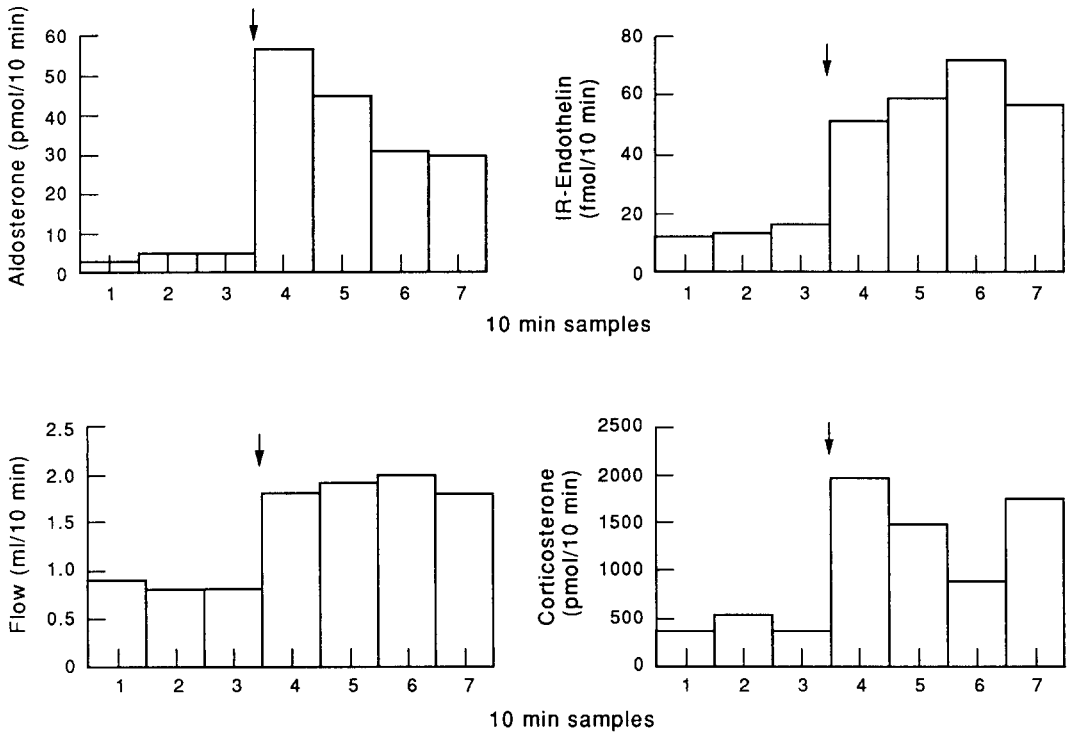

Fig. 5. Effects of 1 nmol histamine (time of administration indicated by the arrow) on flow rate and aldosterone, corticosterone and endothelin secretion by the isolated perfused rat adrenal gland *in situ*. In a series of 5 experiments this dose of histamine caused an average $87 \pm 15\%$ increase in flow rate ($P < 0.01$), a $390 \pm 95\%$ increase in aldosterone secretion ($P < 0.05$) and a $450 \pm 100\%$ increase in corticosterone secretion ($P < 0.01$). Data taken from Ref. [23].

Stimulation of blood flow by ACTH

Clearly, there may be several ways in which blood flow can be modulated in any vascular bed. These include direct neural control of arteriolar tone, and it is interesting that peptidergic neurones, containing vasoactive intestinal polypeptide [15], calcitonin gene-related peptide [16], substance P [17] and neuropeptide Y [18, 19], as well as catecholaminergic neurones [20], have been identified in the adrenal cortex, mostly located in the outer part of the gland, in the capsular and subcapsular region; the region in which, in the rat adrenal, vascular tone and blood flow are regulated [9]. The possibility that ACTH might modulate such neural function has not been examined, but cannot be excluded. Alternatively, blood flow could be affected by the local release of vasoactive substances, in a paracrine fashion, and in this connection the identification of an intra-adrenal renin–angiotensin system is of particular interest [21, 22]. Once again, the interaction of ACTH (or of other effectors) with this system has not been examined.

The data presented here, however, strongly indicate a role for mast cells and it is significant that their generally capsular location, near the point at which the afferent arterioles enter the gland [23], is entirely appropriate in this respect. The action of Compound 48/80, a mast cell degranulator, increases perfusion medium flow through the gland, and under the perfusion conditions used, this can be taken to indicate relaxation of vascular tone. These effects are also seen when the system is stimulated with either histamine or serotonin, both of which are mast cell products in the rat [23]. Furthermore,

Fig. 6. Proposed links between ACTH, increased flow rate, and increased steroidogenesis. Mast cells (M) are located near the arterioles (A) in the region of the capsule (CAP) and are stimulated by ACTH to secrete serotonin and histamine (SH). This brings about vasodilation, allowing increased blood flow through the gland. The consequent engorgement of the centripetally orientated sinuses (S), which lie in contact with virtually every adrenocortical cell, stimulates endothelial secretion of endothelin (E), which in turn directly stimulates corticosteroid secretion (CS) by zona glomerulosa (ZG) and zona fasciculata (ZF) cells.

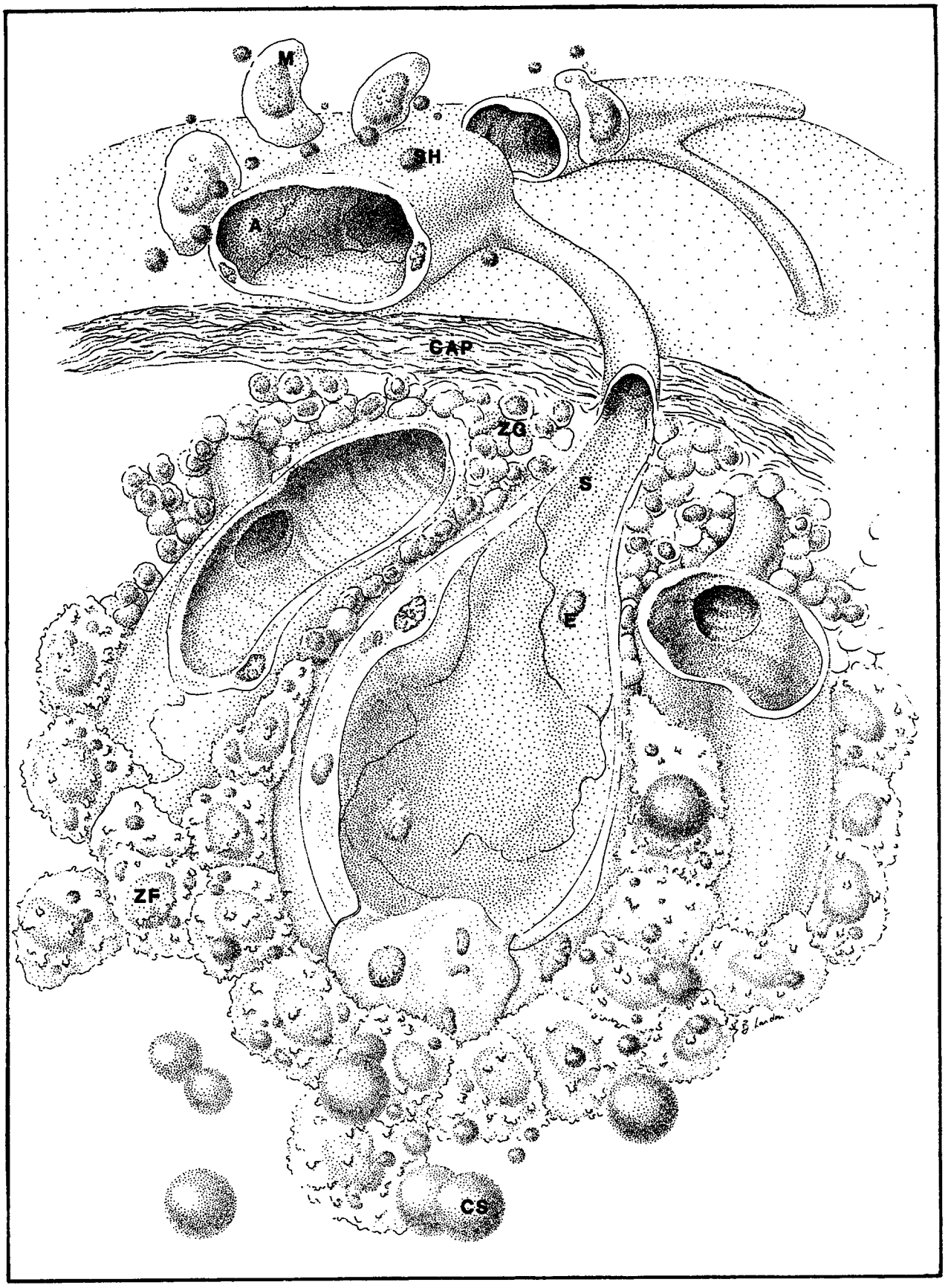

Fig. 6. *Caption opposite.*

sodium cromoglycate, a mast cell stabilizer, markedly inhibits the vascular response to ACTH stimulation. It may be significant that corticosterone, but not aldosterone secretion is also inhibited under these conditions, since it is evident from all of the data that has been obtained using this preparation that inner zone function is correlated with gross flow rate, while zona glomerulosa function is not [12]. In the *in situ* perfused rat adrenal system, corticosterone secretion is a specific indicator of zona fasciculata function, and is not secreted by the glomerulosa. However, the situation may be more complex because aldosterone secretion may also be increased by agents which affect the vasculature which is undoubtedly stimulated by increased flow rate (see below).

These data do not exclude a role for the other possible mechanisms listed above, and it is highly probable that the various reports of the effects on flow of splanchnic nerve stimulation (or section) in other species [24], or of field stimulation in the rat [25], are mediated through the direct innervation of the vasculature. However, the important aspect of the present findings is that, for the first time, a mechanism for the regulation of blood flow *by ACTH*, and perhaps of other stimulants not known to act through neuromodulation, can be deduced.

Stimulation of steroidogenesis by increased flow

It might be tempting to suppose there are various simple explanations for increased steroidogenesis when flow through the gland is increased, for example, through increased supply of oxygen, or the rapid removal of toxic products, or indeed of steroids. Were this the case, however, it would be expected that exactly the same effects should be seen when dispersed adrenocortical cells are superfused at different rates. This does not occur, and therefore it is evident that other components of the adrenal gland, not present in the dispersed cell preparation, must modulate these effects [12]. Clearly there are several such components, and the nervous system could again be invoked. Certainly it has been proposed that some neurotransmitters stimulate adrenal zona fasciculata cells directly [26]. However, direct innervation of the zona fasciculata is very sparse, if it occurs at all, and most of the innervation, as mentioned above, seems to be more clearly associated with the vasculature in the region of the capsule. A humoral modulator seems a more obvious contender.

The vasculature of the adrenal gland is extensive. Thin-walled capillaries (usually called sinuses in the adrenal) pervade the entire gland, and virtually every adrenocortical cell lies adjacent to endothelial tissue [2]. The endothelium is probably the largest endocrine gland in the body, and it seems highly plausible that endothelial products could affect adrenocortical function. The actions of endothelin on aldosterone secretion have already been described, and endothelin receptors have been characterized in the zona glomerulosa [27, 28]. The present results show that in addition, endothelins stimulate rat adrenal dispersed fasciculata cells, with high sensitivity, and indeed are the only naturally occurring peptides other than ACTH known to do so. Importantly, the results also show that the secretion of immunoreactive endothelin into the adrenal vein is significantly increased when steroidogenesis and flow are stimulated by either ACTH, or by histamine, an agent which is a potent stimulator of steroidogenesis in the intact gland, but which is entirely without effect on steroid secretion by collagenase-dispersed adrenocortical cells [23]. This observation strongly suggests that the increase in perfusate flow following vasodilation, and the consequent increase in corticosterone secretion may be linked by the release of endothelin from the vascular endothelium.

A summary of the proposed links between ACTH, vascular events and steroidogenesis is illustrated in Fig. 6. The amplification of the ACTH signal in the intact gland in this way may provide an explanation for the vastly greater amounts of corticosterone which the intact gland can produce, compared with any *in vitro* preparation, and also for the differences in responsiveness of the intact adrenal gland compared with *in vitro* systems.

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