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THE FAILURE OF INDOMETHACIN TO ALTER ACTH-INDUCED ADRENAL HYPERAEMIA OR STEROIDOGENESIS IN THE ANAESTHETIZED DOG

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1 The response of adrenal blood flow to adrenocorticotropic hormone (ACTH) was measured with radioactive microspheres in anaesthetized, dexamethasone-treated, mongrel dogs.

2 Adrenocorticotropic hormone (2 u/h i.v.) increased adrenal blood flow within 15 min and this persisted for the duration of the infusion.

3 Cortisol concentrations also rose with ACTH infusion.

4 Indomethacin (6 mg/kg i.v. followed by 1 mg/min) did not affect the adrenal response to ACTH although plasma concentrations of indomethacin (21.9 ± 2.5 μg/ml) adequate to suppress prostaglandin synthesis were achieved.

5 We conclude that prostaglandins are not required for steroidogenesis or the adrenal haemodynamic response to ACTH.

Introduction

Prostaglandins are released from adrenal cortical cells of the rat and cat by adrenocorticotropic hormone (ACTH). The precursor of the prostaglandins, arachidonic acid, is present in abundance in the adrenal gland, esterified in phospholipids and cholesterol esters (Goodman, 1965; Laychock, Shen, Carmines & Rubin, 1978). Hydrolysis of the cholesterol esters by ACTH results in the simultaneous release of cholesterol for steroid biosynthesis and arachidonic acid, which is converted in part to prostaglandins (Vahouny, Chanderbhan, Hodges & Treadwell, 1978).

Although several investigators have hypothesized a functional role of prostaglandins in mediating or modulating steroid biosynthesis by influencing adrenal cyclic adenosine 3',5'-monophosphate (cyclic AMP) concentrations (Flack, Jessup & Ramwell, 1969; Saruta & Kaplan, 1972; Gallant & Brownie, 1973; Warner & Rubin, 1975; Laychock & Rubin, 1976; Honn & Chavin, 1977), there is little evidence that prostaglandins are indeed important in steroidogenesis. Another postulated role for prostaglandins in the adrenal and other organs is as a regulator of the circulation (Staszewska-Barczak & Vane, 1975; Maier & Staehelin, 1968a, b). The factors controlling the adrenal circulation are largely unknown because of technical difficulties in measuring adrenal blood flow. However, stresses such as haemorrhage or endotoxin shock which result in adrenal steroid release also increase adrenal blood flow in the primate, dog and rat (Frank, Frank, Jacob, Weizel, Korman & Fine, 1956; Sapirstein, Sapirstein & Bredemeyer, 1969; Wyler, Forsyth, Nies, Neutze & Melmon, 1969; Forsyth, Hoffbrand & Melmon, 1970). ACTH likewise has been reported to result in adrenal hyperaemia (Maier & Staehelin, 1968a, b). We proposed a hypothesis that ACTH stimulates hydrolysis of the cholesterol arachidonate esters with the arachidonate being formed into vasodilatory prostaglandins that mediate an increase in adrenal blood flow. We tested this hypothesis in the dog by the use of the radioactive microsphere method for determining blood flow and of indomethacin to inhibit prostaglandin synthesis.

Methods

Thirteen mongrel dogs of either sex weighing 20 to 30 kg were divided into a control ($n = 6$) and indomethacin ($n = 7$)-treated group. All dogs were pretreated with dexamethasone 5 mg intramuscularly the night before and the morning of the experiment to suppress endogenous ACTH. Dogs were anaesthetized with sodium pentobarbitone (25 mg/kg i.v.) and respired through an endotracheal tube connected to a Harvard respirator. Polyethylene cannulae were placed in the femoral artery, both femoral veins and the left ventricle via a carotid artery. Dogs treated with indomethacin recieved an initial intravenous

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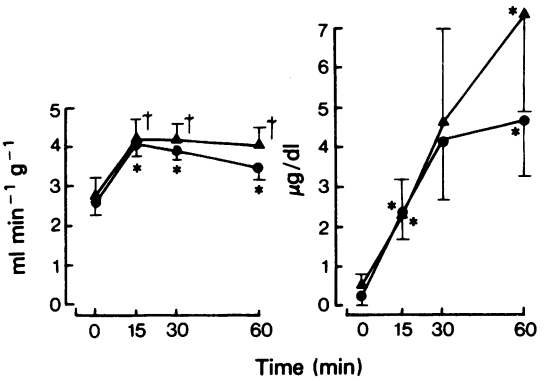

Figure 1 Effects of ACTH on (a) adrenal blood flow and (b) plasma cortisol concentration (mean of 6 (control) or 7 (indomethacin-treated)). ACTH was given at 2 u/h intravenously beginning at time zero. A significant rise from the zero time is indicated by * ($P < 0.05$) and † ($P < 0.01$). (●) Without indomethacin treatment; (▲) with indomethacin treatment. There were no significant differences between the indomethacin and control groups at any time.

bolus of 6 mg/kg in sodium carbonate buffer followed by a continuous intravenous infusion of 1 mg/min for the duration of the experiment. ACTH (Acthar, Armour Pharmaceutical Co., Phoenix, Arizona) was infused at 2 u/h intravenously for 60 min. Arterial pressure and heart rate were monitored continuously. At 0, 15, 30 and 60 min after the start of the ACTH infusion, 500,000 to 1,000,000 radioactive microspheres (15 ± 3 μm, 3 M Co., St. Paul, MN) labelled with ^{51}Cr, ^{85}Sr, ^{141}Ce, ^{95}Nb were injected into the left ventricle and a reference sample of arterial blood obtained for cardiac output determination as described previously (Archie, Fixler, Ullyot, Hoffman, Utley & Carlson, 1973). At the termination of the experiment, the dogs were killed and the adrenal glands, kidneys and spleen dissected, weighed and placed in counting vials for determination of radioactivity in an Auto Gamma spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). The methods for determining cardiac output and organ blood flows with radioactive microspheres have been published (Rudolph & Heymann, 1967; Archie *et al.*, 1973).

Samples of arterial blood were obtained at 0, 15, 30 and 60 min for plasma cortisol determination by radioimmunoassay (Clinical Assays, Cambridge, MA). A sample of blood taken at 60 min was analyzed for indomethacin concentration by high pressure liquid chromatography. Three μl of plasma was injected without extraction onto a C-18-bondapak column (Waters, Inc., Milford, MA) and the indomethacin eluted isocratically with 50% acetonitrile in 0.01 M nitric acid at a flow rate of 2 ml/min with detection by u.v. absorption at 254 nm. Data were analyzed by Student's *t* test for paired observations (changes from baseline with ACTH) and by Student's *t* test for unpaired observations (difference between control and indomethacin groups).

Results

ACTH administration consistently increased adrenal blood flow within 15 min and flow remained elevated for the duration of the infusion (Figure 1). Blood flow to the other organs examined, spleen and kidney, did not increase indicating the specificity of the effect of ACTH (Table 1). Cardiac output and mean arterial pressure were stable throughout the experiment (Table 1). Indomethacin pretreatment produced no change in adrenal blood flow response to ACTH.

The concentration of indomethacin in the plasma at the conclusion of the experiment was 21.9 ± 2.5 μg/ml.

Table 1 Effects of ACTH on systemic and regional haemodynamics

		Time (min after start of ACTH infusion)			
		0	*15*	*30*	*60*
Cardiac output	Control	3436 ± 143	3227 ± 322	3216 ± 243	3207 ± 486
(ml/min)	Indo	3978 ± 697	4022 ± 852	3299 ± 491	3618 ± 719
Mean arterial	Control	156 ± 5	156 ± 5	157 ± 5	158 ± 5
pressure (mmHg)	Indo	157 ± 5	157 ± 4	156 ± 3	156 ± 4
Renal blood flow	Control	5.7 ± 0.7	5.5 ± 0.4	5.6 ± 0.4	5.4 ± 0.5
ml min^{-1} g^{-1}	Indo	5.7 ± 0.4	5.6 ± 0.5	5.4 ± 0.5	5.5 ± 0.5
Splenic blood flow	Control	1.9 ± 0.3	1.9 ± 0.3	1.9 ± 0.3	1.9 ± 0.4
ml min^{-1} g^{-1}	Indo	1.9 ± 0.3	2.0 ± 0.4	1.6 ± 0.3	1.8 ± 0.4

Each value is the mean of 6 (control) or 7 (indomethacin-treated) determinations and is shown with its standard error. Indo = indomethacin-treated dogs.

Discussion

A functional role for prostaglandins produced by the adrenal gland has been the subject of speculation and experimentation for over a decade (Maier & Staehelin, 1968a, b; Flack *et al.*, 1969; Saruta & Kaplan, 1972; Gallant & Brownie, 1973; Warner & Rubin, 1975; Laychock & Rubin, 1976; Honn & Chavin, 1977). There seems to be little doubt that adrenal cortical cells produce prostaglandins E_2 and $F_{2\alpha}$ upon stimulation with ACTH, and the precursor pool of arachidonic acid for prostaglandin synthesis appears to be the cholesterol esters in the adrenal gland (Vahouny *et al.*, 1978). The cholesterol liberated by the action of ACTH is used for steroid biosynthesis, and the arachidonic acid is used for prostaglandin and phospholipid biosynthesis. It is tempting to think that the prostaglandins produced in this way are more than byproducts of steroidogenesis. Two proposed roles for adrenal prostaglandins have been as a second messenger for steroid biosynthesis (Warner & Rubin, 1975) and as a regulator of adrenal blood flow during ACTH stimulation (Maier & Staehelin, 1968b).

However, there is no conclusive evidence *in vivo* or *in vitro* to support a functional role for prostaglandins and our data give no support to either of the postulated roles. We found that ACTH produces adrenal hyperaemia in the anaesthetized dog coincident with steroidogenesis. Neither of these effects of ACTH were modified in any way by indomethacin, a potent inhibitor of prostaglandin cyclo-oxygenase. It might be argued that the amount of indomethacin we administered was insufficient to suppress the ACTH-induced adrenal production of prostaglandins. We have no direct way to refute this argument since we cannot measure the output of adrenal prostaglandins in the intact animal. However, we think it unlikely that the concentration of indomethacin was insufficient to inhibit prostaglandin cyclo-oxygenase in the adrenal gland. The doses of indomethacin used are adequate to inhibit prostaglandin synthesis in the kidney (Nies, Rawl, Cruze, Oates & Frölich, 1978), pregnant uterus (Gerber, Branch, Hubbard & Nies, 1978), and heart (Hintze & Kaley, 1977) of the dog. Concentrations of indomethacin in excess of that needed in man to inhibit platelet and total body synthesis of prostaglandins (Rane, Oelz, Frölich, Seyberth, Sweetman, Watson, Wilkinson & Oates, 1978) were achieved in our experiments. It is not necessarily valid to translate directly the results of experiments done *in vitro* to the situation *in vivo*. Nevertheless, it is worth mentioning that concentrations of indomethacin were much in excess of those needed (0.1 μg/ml) to inhibit ACTH-induced prostaglandin release *in vitro* from adrenal cortical cells of the cat (Laychock & Rubin, 1976), even accounting for protein binding of indomethacin (~90% in man; Mason & McQueen, 1974).

The radioactive microsphere method for determining adrenal blood flow gave very consistent results in these experiments and could be a useful method to explore further the control of adrenal haemo-dynamics *in vivo* with minimal surgical intervention. Other methods for measuring blood flow are difficult to apply to the adrenal because of its small size and multiple blood supply. The major requirement for the radioactive microsphere method is the presence of sufficient numbers of microspheres in the tissue of interest so that random variability in distribution of the microspheres is minimized. Buckberg, Luck, Payne, Hoffman, Archie & Fixler (1971) determined that at least 400 microspheres were required for acceptable results. With adrenal blood flow accounting for about 0.2% of the cardiac output in our dogs, we estimate that at least 1000 microspheres were trapped by the adrenal glands in our experiments.

Prostaglandins in the adrenal cortex may have a role in the production of aldosterone, which we did not examine. It is also conceivable that an effect of indomethacin might be detected at smaller doses of ACTH. If this were the case, prostaglandins might be a subtle modulator of some of the effects of ACTH. However, it appears from our data that the increase in adrenal blood flow and in steroid production caused by ACTH *in vivo* are not dependent upon prostaglandins. A similar conclusion was reached by Laychock & Rubin (1977) regarding the necessity of prostaglandins for ACTH-induced steroidogenesis in cat adrenocortical cells *in vitro*. In conclusion, although the production of prostaglandins by the adrenal is stimulated by ACTH, the function, if any, of these prostaglandins remains unknown. They are not required for ACTH-induced changes in adrenal haemodynamics or glucosteroidogenesis.

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