Effects of dopamine, high potassium concentration and field stimulation on the secretion of aldosterone by the perfused rat adrenal gland

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ABSTRACT

The rat adrenal cortex contains quantities of dopamine that are compatible with its function as a neurotransmitter, suggesting that locally released dopamine may act as a neurotransmitter within the gland. This possibility has been tested by comparing the effects of dopamine on aldosterone secretion in the perfused adrenal with the effects of stimuli designed to provoke the release of intraglandular dopamine.

Infusion of dopamine (0.1–100 μmol/l for 10-min periods) into the isolated perfused rat adrenal gland resulted in a transient, dose-related reduction of aldosterone secretion to a minimum of approximately 50% of the basal value at 1 μmol dopamine/l (ratio of experimental to control measurements, R = 0.53 ± 0.06 (S.E.M.); n = 5). In contrast, dopamine (1–100 μmol/l) had no effect on aldosterone production by dispersed zona glomerulosa cell preparations incubated in vitro.

The effects of changes in K+ concentration (3.9–52 mmol/l) on aldosterone secretion in the perfused gland and dispersed cell preparations were also compared. A similar bell-shaped dose–response relationship was seen in both preparations between 6 and 32 mmol K+/l, with a maximum at 8.4 mmol K+/l and a return to control values with 16, 24 or 32 mmol K+/l. However, infusion of media with very high K+ concentrations (42 or 52 mmol K+/l) reduced the secretion of aldosterone by the perfused gland to approximately 50% of the basal value (R = 0.51 ± 0.05, n = 9; R = 0.49 ± 0.08, n = 9; respectively) but produced no change in aldosterone production by zona glomerulosa cells. Electrical field stimulation (pulse width 1 ms, 1 Hz at 60 V for 5 min) of the perfused gland also resulted in a reduction in aldosterone secretion (R = 0.66 ± 0.06, n = 6). In the presence of 1 μmol haloperidol/l, a dopamine antagonist, no effect on aldosterone secretion was seen under control conditions, but the responses to 1 μmol dopamine/l, 52 mmol K+/l and field stimulation were eliminated.

The results are consistent with the view that aldosterone secretion by the perfused adrenal gland is subject to an inhibitory dopaminergic control, which may originate from catecholaminergic neurones within the gland itself.

Journal of Endocrinology (1992) 133, 275–282

INTRODUCTION

The secretion of aldosterone from the zona glomerulosa of the rat adrenal cortex is known to be under multifactorial control, and particular attention has been focused on the role of angiotensin II, potassium ion (K+) concentration and corticotrophin (Tait, Tait & Bell, 1980a; Müller, 1988). In recent years additional regulators have been proposed, including dopamine which has been suggested to exert a tonic inhibitory influence on aldosterone secretion (Carey, Thorner & Ortt, 1979; Sowers, 1984). This concept arose from the observation that treatment with bromocriptine (a dopamine agonist) attenuates the stimulation of aldosterone secretion produced by the diuretic, furosemide, and that metoclopramide (a dopamine antagonist) increases plasma aldosterone concentration in man (Edwards, Thorner, Miall et al. 1975; Norbiato, Bevilacqua, Raggi et al. 1977). These actions appear to be independent of changes in the renin-angiotensin system (Sowers, Tuck, Golub & Sollars, 1980; Carey, 1982). However, there has been some debate about the validity of this interpretation and it has been suggested that the effects of metoclopramide are...
indirect. The drug interacts with other receptors and affects the secretion of several hormones from the pituitary, which may themselves affect aldosterone secretion (Campbell, Mendelsohn, Adam & Funder, 1981; McDougall, Scoggins, Butkus et al. 1981; Whitehouse, Vinson & Thody, 1982; Braley, Menachery & Williams, 1983; Fraser, 1987). Moreover, although there is some evidence that dopamine can directly affect adrenal function (see Fraser, Connell, Inglis et al. 1989), the levels required are considerably higher than the concentrations of dopamine which occur naturally in plasma (Van Loon & Sole, 1980).

A further area of debate concerns the origin of the dopaminergic tone. Dopamine, a catecholamine, is not only a precursor of noradrenaline and adrenaline but is well characterized as a transmitter in the central and peripheral nervous system (Hornykiewicz, 1966; Lackovic & Neff, 1980). Specific dopamine receptors of both the DA-1 and DA-2 subclasses have been detected in the rat adrenal cortex (Dunn & Bosman, 1981; Bevilacqua, Vago, Scorza & Norbiato, 1982; Missale, Liberini, Memo et al. 1986; Stern, Tuck, Ozaki & Krall, 1986). It has been suggested that dopamine may function as a neurohormone within the adrenal cortex: significant quantities are found within the tissue (although it may not be synthesized there) and the dopamine/noradrenaline ratio is comparable with sites where the amine is an established neurotransmitter (McCarty, Kirby & Carey, 1984, 1986; Racz, Buu & Kuchel, 1984a; Inglis, Kenyon, Hannah et al. 1987; Buu & Lussier, 1990). Catecholaminergic fibres have been detected in the cortex which enter with vascular plexuses, branch into the zona glomerulosa and are dispersed among the adrenocortical cells (Kleitman & Holzwarth, 1985). These fibres could be eliminated by chemical sympathectomy but not by cutting the splanchic nerve. However, there is no direct evidence that dopamine is contained within the nerve terminals.

We have adopted an alternative approach to investigate the possible paracrine role of dopamine in the control of aldosterone secretion in the rat by studying responses in the perfused adrenal. This technique allows orthograde perfusion of the adrenal, with its circulation isolated from the rest of the body and from the influences of the pituitary or kidney. Importantly, the adrenal vasculature, the various cells and their relationship with each other remain undisturbed and it is possible to test the effects of agents on the intact gland under defined conditions (Sibley, Whitehouse, Vinson et al. 1981; Hinson, Vinson, Whitehouse & Price, 1985). Using this technique, it has been possible to show sensitive responses to neurotransmitters and to provide evidence in favour of paracrine control of adrenocortical function (Porter, Whitehouse, Taylor & Nussey, 1988; Hinson, Vinson, Whitehouse & Porter, 1989b). In addition, it is possible to stimulate excitable cells in the gland by using electrical stimulation or high (depolarizing) concentrations of K+ (Porter & Whitehouse, 1986). We have, therefore, attempted to provoke the release of intraglandular dopamine with these techniques and compared their effects with that of exogenous dopamine in the perfused rat adrenal.

**MATERIALS AND METHODS**

**In-situ perfusion of rat adrenal gland**

Male Sprague–Dawley rats (250–450 g), raised and housed in the Kensington Campus Animal Unit of King’s College London, were anaesthetized with 1·4 g/kg i.p. urethane (Sigma Chemicals, Poole, Dorset, U.K.) and heparin (100 units in 1 ml 0-9% (w/v) saline) was administered via a cannula in the left jugular vein. The left adrenal gland was prepared for in-situ perfusion by the method of Sibley et al. (1981) which allows orthograde perfusion of the undisturbed adrenal gland by an artificial medium. Briefly, perfusate is introduced into an isolated segment of the aorta via a cannula in the coeliac artery and, after passing through the adrenal, it is collected from a pocket in the renal vein, so that the gland is perfused without interference from the animal’s blood. In all experiments a recovery period of 30 min during which the medium was discarded preceded the experiment during which 10-min collections of perfusate were made. The perfusion medium used was Tissue Culture Medium 199 (GibCo Ltd, Paisley, Strathclyde, U.K.) at 37 °C, pH 7·2, plus 0-5% bovine serum albumin (Type V, Sigma), normally diluted 4·3 with KCl-free Krebs–Ringer bicarbonate solution to give K+ and Na+ concentrations of 3·9 and 146 mmol/l respectively (medium PM1). The effects of dopamine hydrochloride (3-hydroxytyramine hydrochloride plus 0-4% ascorbate; Sigma) and medium with 6, 8·4, 16, 24, 32, 42 or 52 mmol K+ l were tested. The media with increased K+ concentration were prepared by mixing Medium 199 with appropriately modified Krebs bicarbonate Ringer, in which the Na+ content was reduced as the K+ content was increased in order to maintain osmolarity. Normally, test substances were infused for 10 min after a control period of 40–60 min; in some experiments the dopamine antagonists, haloperidol or sulpiride (1 μmol/l; Sigma), were also added to the medium.

The gland was stimulated electrically by resting a positive silver electrode on its surface and placing a negative electrode on adipose tissue about 0·75 cm away. Stimulation parameters were taken from Wakade (1981): 1 Hz at 60 V with a pulse width of

*Journal of Endocrinology* (1992) 133, 275–282
1 ms using a Palmer Square-Wave stimulator for 5 min. This technique permits stimulation of the presynaptic splanchnic nerve terminals within the gland as shown by the release of adrenaline and noradrenaline from the adrenal medulla (Wakade, 1981; I. D. Porter & B. J. Whitehouse, unpublished observations). No effect on steroid secretion or flow of perfusate was evident with the electrode simply touching the gland.

Dispersed cell incubations

This technique has been described in detail previously (Vinson, Whitehouse, Bateman et al. 1986; Purdy, Whitehouse & Abayasekara, 1991). In brief, adrenals were rapidly removed from freshly killed female Sprague–Dawley rats and stored briefly in saline on ice. Cells suspensions were prepared by incubating adrenal capsules with collagenase (2 mg/ml; Worthington Type 1, Cambridge Bioscience, Cambridge, Cambs, U.K.) in medium PM1 (as described above) with 1% BSA for 1 h. Cells were dispersed by repeated pipetting and washed in fresh medium and incubated for 2 h at a density of 10 000 cells/ml in medium PM1 with dopamine (1-100 μmol/l) or in medium of 6, 8-4, 16, 24, 32, 42 and 52 mmol K+/l (as described above). The viability of the cells was tested by including a batch incubated with 1 nmol ACTH(1-24)/l (Synacthen, Ciba-Geigy, Horsham, Sussex, U.K.) in each experiment.

Estimation of aldosterone

All samples were made up to 5 ml with distilled water, washed with 2-5 ml n-hexane (HPLC grade: Fisons, Loughborough, Leics, U.K.), and extracted twice with 2-5 ml ethyl acetate (HiPersolv; BDH Chemicals Ltd, Poole, Dorset, U.K.). After evaporation of the solvent, aldosterone was estimated by radioimmuno-assay (Vazir, Whitehouse, Vinson & McCredie, 1981).

Analysis of data

Results are shown as means ± s.e.m. and expressed as the ratio of experimental to control measurements (R). This was calculated for the perfusion experiments as the mean of two aldosterone secretion rates measured after the administration of substance divided by the mean of two basal rates sampled immediately before. For dispersed cell incubations, R was calculated as the ratio of aldosterone production in control incubations (3-9 mmol K+/l medium) to that in experimental incubations. Statistical significance is assessed by Student's t-test or analysis of variance (ANOVA), as appropriate.

RESULTS

Effects of dopamine on aldosterone secretion from the rat adrenal cortex

A typical response for the perfused gland to 10-min infusions of 1-0 μmol dopamine hydrochloride/l in the perfused gland is shown in Fig. 1, which reveals a brief decrease in aldosterone secretion following each exposure to dopamine. In a series of similar experiments, dopamine (1 μmol/l) reduced aldo¬

Stimulation

FIGURE 1. Typical effects of 1 μmol dopamine/l on aldosterone secretion by a perfused adrenal gland. Dopamine was added to the perfusion medium for 10-min periods indicated by the solid horizontal bars.

Effects of changes in K+ concentration and electrical field stimulation

Complete dose–response curves for changes in aldosterone secretion with changes in K+ concen¬

Stimulation

tration from 3-9 to 52 mmol/l in the perfused gland and capsular cells are shown in Fig. 3. In order to maintain the osmolarity of the media the Na+ concen¬

tration was reduced proportionally from 146 to 98 mmol/l. It can be seen that the responses of both types of preparation are similar up to a concentration of 32 mmol K+/l, but diverge at 42 and 52 mmol K+/l. A small increase in K+ concentration to 84 mmol/l caused the maximum stimulation of aldosterone
secretion from both the perfused gland (\(R = 2.5 \pm 0.4\); \(n = 7\)) and from dispersed cells in vitro (\(R = 5.65 \pm 2.0\); \(n = 3\)). No significant stimulation of aldosterone secretion was seen in either preparation between 16 and 32 mmol K\(^+\)/l. However, at 42 and 52 mmol K\(^+\)/l, aldosterone secretion from the perfused gland was decreased to approximately half the basal rate (\(R = 0.51 \pm 0.05\), \(n = 9\); \(R = 0.47 \pm 0.08\), \(n = 9\); respectively), whereas production by the cellular preparation was no different from that at 3.9 mmol K\(^+\)/l. In addition, there was no difference between the response of zona glomerulosa cells to stimulation with 1 mmol ACTH(1-24)/l in 3.9 mmol K\(^+\)/l and 52 mmol K\(^+\)/l (\(R = 6.5 \pm 0.7\) and 5.9 \(\pm 0.6\) respectively). A typical response of an individual gland to periods of perfusion of 52 mmol K\(^+\)/l medium is shown in Fig. 4a. Reduction of Na\(^+\) content of the medium to 98 mmol/l without increasing K\(^+\) content by substitution of sodium chloride by choline chloride (48 mmol/l) did not reduce the output of aldosterone from the perfused gland (data not shown).

The response to field stimulation (electrical stimulation at 60 V, 1 ms and 1 Hz for 5 min) was similar to that obtained with 42 or 52 mmol K\(^+\)/l with a transient decrease in aldosterone secretion from the perfused gland following the period of stimulation (Fig. 4b). Data summarizing the results of six similar experiments are shown in Fig. 5 (for the effects of field stimulation \(R = 0.66 \pm 0.06\); \(n = 6\); \(P < 0.01\)).

**Effect of dopamine antagonists**

The results of experiments in which the dopamine antagonist, haloperidol (1 \(\mu\)mol/l), was added to the perfusion medium are summarized in Fig. 5, where it can be seen that the drug did not affect aldosterone output from the preparation under control conditions. However, when haloperidol was infused during the application of dopamine, the previously recorded reduction in aldosterone secretion was eliminated, as was the response to 52 mmol K\(^+\)/l and field stimulation. In addition, infusion of a further dopamine antagonist, sulpiride (1 \(\mu\)mol/l), eliminated the reduction of aldosterone secretion produced by 42 mmol K\(^+\)/l (\(R = 0.55 \pm 0.11\) and 1.13 \(\pm 0.12\) respectively, \(n = 3\); \(P < 0.05\)).

**DISCUSSION**

Controversy has long surrounded the reported effects of dopamine on adrenal function, and many inconsistencies are apparent when comparisons between different species and preparations are made (Campbell et al. 1981; Fraser et al. 1989). For instance, infusion of dopamine into the auto-transplanted adrenal of sheep had no effect on either basal or angiotensin II-stimulated aldosterone secretion (McDougall et al. 1981). In human subjects, the decreased aldosterone response to angiotensin II has been attributed to increased clearance rates of the infused peptide rather than a direct effect on the adrenal cortex (Connell, Tonolo, Davies et al. 1987). Studies with dopamine in vitro have yielded inconsistent results, with inhibition of potassium-, ACTH- and angiotensin II-stimulated aldosterone secretion being reported by some authors.
FIGURE 4. Typical effects on aldosterone secretion by a perfused adrenal of (a) increasing the K⁺ concentration of the perfusion medium to 52 mmol/l for 10-min periods (indicated by the solid bars) and (b) field stimulation (60 V, 1 ms and 1 Hz for 5 min, indicated by the solid horizontal bar).

FIGURE 5. The effects of 1 µmol haloperidol/l (hatched bars) on aldosterone secretion by perfused adrenal preparations under control conditions and on responses to 1 µmol dopamine/l (DOPA), field stimulation (FS; 60 V and 1 Hz) and 52 mmol K⁺/l medium (K⁺). The data are shown as the ratio of experimental to control values. *Significantly different from perfusions without haloperidol (n = 3; unpaired Student's t-test; P < 0.05 or better).
nor ACTH-stimulated aldosterone secretion by rat zona glomerulosa cells was affected (Fig. 4). In addition, in perifusions of mouse adrenal cells, the response to ACTH was potentiated by 53 mmol K+ /l medium (Robertson, Keith & Kendall, 1984). It seems reasonable, therefore, to assume that the reduction of aldosterone secretion in the perfused gland is secondary to the release of intraglandular mediators and caused by the depolarization of excitatory tissues (e.g. nerve endings or paracrine cells). Further evidence to support this view is provided by the fact that electrical field stimulation of the perfused adrenal also resulted in a reduction in aldosterone secretion (Fig. 4b and Fig. 5). The technique of transmural stimulation of the adrenal used in these experiments was originally used to excite presynaptic splanchnic nerve endings and provoke secretion of catecholamines from the perfused gland (Wakade, 1981; Sharma, Wakade, Malhotra & Wakade, 1986). Direct stimulation of the splanchnic nerve in the calf has been shown to release small amounts of dopamine and several peptides, in addition to adrenaline and noradrenaline (Allen, Bircham, Bloom & Edwards, 1984; Bloom, Edwards & Jones, 1988; Edwards, 1988).

Insight into the possible nature of the inhibitory modulator bringing about the reduction in aldosterone secretion in the perfused preparation arises from the experiments with haloperidol. This dopamine antagonist with mixed DA-1 and DA-2 receptor activity eliminated the responses to field stimulation and 52 mmol K+ /l medium (Fig. 5). Taken with the knowledge that dopamine at levels that are compatible with an action as a neurotransmitter (1 μmol/l) is capable of reducing aldosterone secretion in the perfused adrenal, this suggests a scheme in which dopamine is released as a result of depolarization and acts as an intraglandular modulator of aldosterone secretion. The loss of the response to 42 mmol K+ /l in the presence of sulpiride, a more specific DA-2 receptor antagonist, is also consistent with this. However, if dopamine is a neurotransmitter in the adrenal cortex and released as a result of electrical stimulation or perfusion with high K+ , it seems reasonable to expect the dispersed cells and the intact gland to show a similar sensitivity to its inhibitory effects. This is not the case, which may be explained by the relative degree of dopamine interaction with DA-1 and DA-2 receptors as discussed above. Alternatively, as suggested by Aguilera & Catt (1984), the effects of dopamine in the rat adrenal cortex may not be direct but instead mediated by stimulating the release of another inhibitory mediator within the gland, possibly somatostatin, which has been localized in the zona glomerulosa (Aguilera, Harwood & Catt, 1981). During electrical stimulation of the adrenal gland or perfusion with high K+ media it is probable that a number of substances are released, that some may inhibit steroidogenesis whilst others promote it, and that they may affect each other's release. Considering that dopamine is a major inhibitory neurotransmitter in the central nervous system, it seems possible that in the adrenal cortex it may modulate the release of other substances, including acetylcholine, noradrenaline, vasoactive intestinal peptide, Met-enkephalin, neuropeptide Y, corticotrophin-releasing factor, serotonin and histamine, all of which may have a role in the control of adrenocortical steroidogenesis (Bircham et al. 1984; Bloom et al. 1988; Edwards & Jones, 1988; Porter et al. 1988; Hinson, Vinson, Pudney & Whitehouse, 1989a; Hinson et al. 1989b). It also seems possible that the reported variability in the responses to dopamine in different preparations may be attributed to variations in the relative importance of these different elements in adrenocortical control.

In conclusion, the results obtained in this study are consistent with the view that aldosterone secretion by the perfused rat adrenal gland is subject to an inhibitory dopaminergic control. This may originate from catecholaminergic neurones within the gland itself or alternatively from the rays of medullary tissue which are found within the cortex (Gallo-Payet, Pothier & Isler, 1987). The significance of this control system in modulating aldosterone secretion under different physiological conditions remains to be determined.

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REFERENCES


Journal of Endocrinology (1992) 133, 275–282


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