Differential effects of endogenous and exogenous nitric oxide on the release of endothelin-1 from the intact perfused rat adrenal gland in situ

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Abstract Studies using an inhibitor of nitric oxide (NO) synthesis have suggested that endogenous NO may have a role in regulating endothelin release. We investigated the effect of endogenous and exogenous nitric oxide (NO) on the release of irET-1. L-NAME stimulated, but L-arginine inhibited irET-1 release. Perfusing sodium nitroprusside (SNP), however, did not inhibit irET-1 secretion. CyclicGMP, the second messenger for NO action, was stimulated by SNP but not by L-arginine. These data demonstrate that endogenous NO inhibits of irET-1, in a manner which is independent of cGMP, and suggest that this action may contribute to the vasodilatory effect of NO.

Key words: Endothelin-1; Nitric oxide; Adrenal

1. Introduction

Nitric oxide (NO) and endothelin-1 (ET-1) are both endothelium-derived vascular mediators which exert a range of effects on different tissues, including the adrenal cortex. We and others have shown that NO has an important role in the maintenance of adrenal vascular tone, particularly under basal conditions [1,2]. It has also been suggested that locally released endothelin has a role in the regulation of adrenal function, including steroidogenesis [3,4]. We have previously demonstrated that ET-1 is released acutely from the perfused rat adrenal gland in response to changes in perfusion medium flow rate through the gland, and that there is a close correlation between flow rate and ET-1 release [5].

Previous studies investigating interactions between NO and ET-1 have demonstrated that one of the effects of endothelin is to cause increased NO release [6,7]. It has also been suggested that NO may have a role in terminating the response to endothelin by altering ET receptor-mediated events [8].

While it is well established that cyclicGMP-dependent vasodilators may influence ET-1 release, much of the research effort has concentrated on the use of cGMP analogues and atrial natriuretic peptides, and there has been little attention paid to NO, which also stimulates cGMP. Some studies have addressed the role of NO by using inhibitors of nitric oxide synthase, the enzyme responsible for NO production, and in general have shown that inhibition of this enzyme results in increased ET-1 release [9,10]. As far as we are aware, no studies have determined the effect of stimulating endogenous NO production on the release of ET-1.

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The present study was designed to directly investigate the role of NO in regulating ET-1 release from the intact perfused rat adrenal preparation. This experimental approach is ideally suited to the study of vascular effects and vascular mediators, and has been used to investigate various aspects of adrenal vascular function and ET-1 release [3,11]. In the present study we have used the perfused adrenal preparation to investigate the effects of endogenous NO on release of ET-1. Two approaches have been used: first perfusing with an L-arginine free medium, supplemented with various concentrations of L-arginine and second, by infusing L-NAME, an inhibitor of NO synthase, in the presence of L-arginine. In addition we have investigated the effects of exogenous NO by perfusing with an NO donor, sodium nitroprusside.

As previous studies have mainly concentrated on relating cGMP to ET-1 release, the secretion of cGMP was also determined in some of the experiments described.

2. Materials and methods

All chemicals were obtained from Sigma, Poole, Dorset, UK, except for the high sensitivity endothelin assay kits and the cGMP assay kits which were purchased from Amersham International plc, Amersham, Bucks UK. Male Wistar rats, body weight 250–350 g, obtained from Charles River, Margate, Kent, UK were maintained at Queen Mary and Westfield College for a minimum of 5 days prior to use. They were fed standard rat chow and water was available ad libitum. Animals were anaesthetised with urethane (140 mg/kg i.p.) before surgery and 100 U heparin was administered via a cannula in the jugular vein.

The method for perfusing the intact rat adrenal gland in situ has been described in detail elsewhere [12,13]. Briefly, perfusion medium was introduced, via the coeliac artery, into an isolated segment of aorta, from which the adrenal arteries arise. After passing through the adrenal the perfusate was collected from a pocket in the renal vein. Once all the ligatures are in place, and the perfusion is established, the animal is killed with an overdose of urethane into the jugular cannula, and thereafter is used only as a platform for the adrenal gland, which remains in situ.

The perfusion medium was either Hank's Balanced Salt Solution (HBSS) which contains no L-arginine, or Tissue Culture Medium 199 (TC199) which contains L-arginine. Both media have a potassium ion concentration appropriate to the human, and in order to adapt these media for use in the rat, were modified by dilution (4:3) with KCl-free Krebs bicarbonate Ringer, to give a final potassium ion concentration of 3.9 mmol/l, approximating to the normal concentration of this ion in the rat. Bovine serum albumin was added to the perfusion medium (0.5 g/100 ml).

In some experiments either sodium nitroprusside or L-arginine was added to HBSS. The final L-arginine concentration in the modified TC199 was 230 μmol/l. The perfusion medium was continuously gassed with 95% O_2/5% CO_2 and delivered at a constant rate of 0.6 ml/min. This is an isolated perfusion preparation and thus the gland receives only the perfusion medium supplied. The gland remains in situ, however, and is supplied by the orthograde route through the normal vasculature. Although the gland only receives perfusion medium, supplied at a constant rate, not all the medium supplied passes through the gland: some is dispersed through adjacent arterioles. In this way the

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preparation is able to respond to changes in vascular resistance within the gland: a decrease in vascular resistance causes an increase in the proportion of the medium supplied passing through the gland. This is monitored by measuring the volume of perfusion medium collected over a ten-minute period. Under basal conditions approximately 30% of the medium supplied passes through the gland. After an initial equilibration period of 40 min, four 10-min control samples were collected. After this control period the experimental procedures were carried out. In some experiments flow rate through the gland was increased mechanically by increasing the rate of delivery of perfusion medium. In other experiments L-NAME, L-arginine or its enantiomer D-arginine was infused. 10-min samples were collected on ice in plastic tubes throughout the experiment. The volume of each sample collected was recorded and an aliquot taken for ET-1 and cGMP assay. Samples were immediately frozen and stored at −70°C for up to one week prior to assay.

2.1. Statistical analysis

Data were expressed as percentage change from control. Results given are the mean percent change from several experiments ± S.E.M. Statistical analysis of the data was performed using Student's paired *t*-test, or analysis of variance, as appropriate.

3. Results

Supplementing HBSS with sodium nitroprusside caused an increase in perfusion medium flow rate through the perfused adrenal gland (Fig. 1), which was accompanied by an increase in immunoreactive ET-1 (irET-1) release (Fig. 2). The addition of L-arginine to HBSS also caused an increase in flow rate but a decrease in irET-1 secretion (Figs. 1 and 2).

In experiments where the rate of delivery of perfusion medium was altered mechanically, there was a significant correlation between flow rate and irET-1 secretion under all conditions used. Addition of L-arginine, the substrate for endogenous NO production, to HBSS caused a significant decrease in the secretion rate of irET-1 (Fig. 3). In addition, when TC199 was used as perfusion medium (containing 230 μmol/l L-arginine) a lower rate of irET-1 release was observed compared to experiments where HBSS was used (Figs. 3 and 4). Addition of L-NAME to perfusion medium TC199 caused a marked increase in the release of irET-1 (Fig. 4). A summary of the effects of altered endogenous NO production on irET-1 release is given in Table 1.

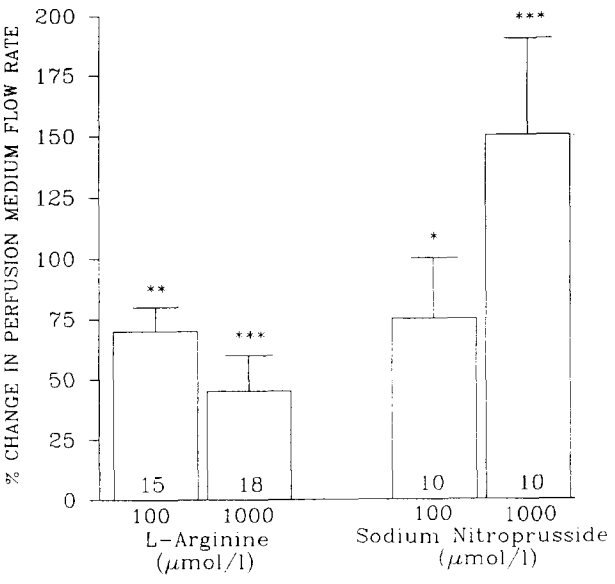

Fig. 1. Percentage increase in perfusion medium flow rate through the intact perfused rat adrenal gland seen by adding either L-arginine or sodium nitroprusside (SNP) to HBSS perfusion medium. Values are mean ± S.E.M. Numbers of experiments are indicated in the bars. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared to basal levels obtained with HBSS alone (analysis of variance).

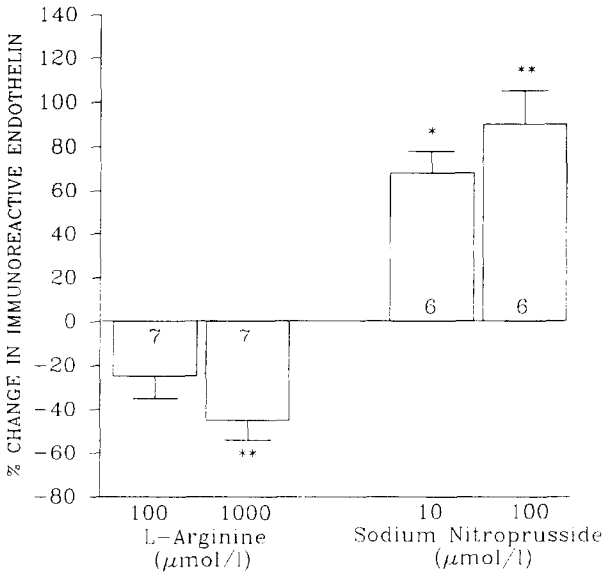

Fig. 2. Effects of adding either L-arginine or sodium nitroprusside (SNP) to HPSS perfusion medium on the release of immunoreactive ET-1 from the perfused rat adrenal gland. Values are mean ± S.E.M. Numbers of experiments are indicated in the bars. $*P < 0.05$, $**P < 0.01$, compared to basal levels obtained with HBSS alone (analysis of variance).

The effects of perfusing with HBSS supplemented with L-arginine and sodium nitroprusside on cGMP release from the perfused adrenal are shown in Fig. 5. While sodium nitroprusside caused a significant stimulation of cGMP release, addition of L-arginine to the perfusion medium significantly attenuated cGMP release. The mean basal rate of cGMP secretion was 550 ± 120 fmol/10 min. D-Arginine had no effect on any of the parameters measured (data not shown).

4. Discussion

The data obtained in the present study, using an intact perfused adrenal gland preparation, demonstrate for the first time that ET-1 release is enhanced in the absence of endogenous NO production, inhibited by endogenous NO, but not affected by the addition of an exogenous NO donor. As most of the experimental studies on endothelial cell function have employed cultured endothelial cells it is difficult to draw direct comparisons between data obtained in this way, and data obtained from an in situ perfusion preparation.

This is the first study, as far as we are aware, which has demonstrated a relationship between NO synthesis and irET-1 release under a variety of different conditions. Previous studies have almost exclusively employed inhibitors of NO synthesis, including L-NAME, and have demonstrated conflicting results, largely depending on the preparation and the stimulus used. Hishikawa and co-workers [14], using cultured endothelial cells, found that the elevated ET-1 secretion seen in response to increased pressure was unaffected by an inhibitor of NO

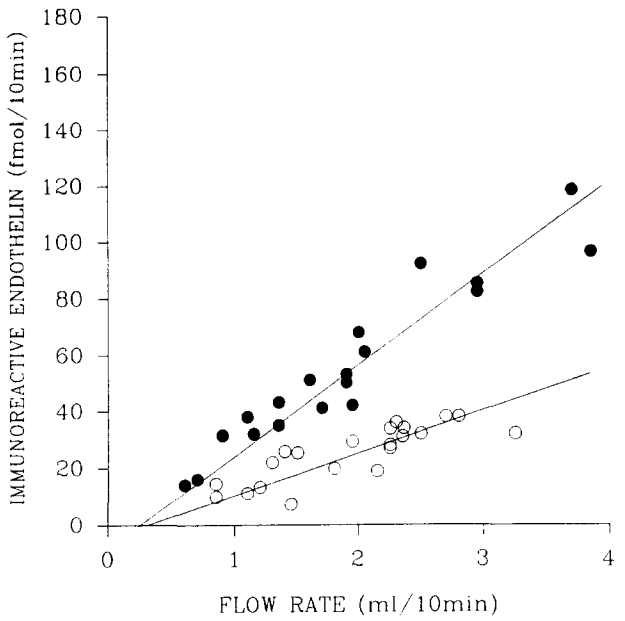

Fig. 3. Effects of perfusing with Hanks balanced salt solution in the presence (○) and absence (●) of L-arginine (1 mmol/l) on the correlation between perfusion medium flow rate and irET-1 release by the intact perfused rat adrenal gland. In each case a highly significant correlation was seen: HBSS alone $r = 0.82$, $P < 0.001$: with L-arginine $r = 0.83$, $P < 0.001$.

synthesis. On the other hand, Boulanger and Luscher [9], using porcine arteries in vitro, found that thrombin-stimulated ET-1 release was enhanced in the presence of an NO synthesis inhibitor, although basal release was unaffected. Cao and co-workers [15] also found that L-NAME increased the ET-1 concentration, measured in the femoral artery of dogs, in response to hypoxia. The present study appears to be the first in which the effects of L-arginine, the substrate for NO synthesis, were investigated. Some other studies have infused L-arginine into human subjects and measured an increase in L-citrulline production, the by-product of NO synthase activity [16]. Presumably the subjects were not L-arginine deficient, but it was, nevertheless possible to stimulate NO synthase activity simply by increasing substrate levels. In the present study, in the experiments using HBSS, the perfusion medium used did not contain L-arginine for the first hour of the perfusion, and thus cellular stores of L-arginine are likely to have been exhausted during this time. The change from L-arginine free medium to L-arginine supplemented medium is likely to cause an increase in NOS activity in this preparation. Therefore it is likely that the effects seen are the result of increased NOS activity.

Different effects of inhibiting NO synthase activity on ET-1 release have been reported depending on whether ET-1 release is basal or stimulated. It is difficult to determine whether the effects reported here should be considered to be effects on basal or stimulated ET-1 release. The addition of L-arginine or sodium nitroprusside to the perfusion medium caused an increase in perfusion medium flow, and it can be assumed that this results in the adrenal endothelial cells being exposed to an increased shear stress. While it has been reported that ET-1 release is stimulated by shear stress [17], this holds true only for low levels of shear stress, with moderate or elevated shear inhibiting ET-1 release while stimulating NO [18]. As we have demonstrated a direct correlation between flow rate through the adrenal gland and irET-1 release it is unlikely that the shear stress generated in these experiments is significantly inhibiting ET-1 release. We have previously shown that the addition of L-NAME to the perfusion medium causes a significant decrease in flow rate through the gland [1], and is therefore unlikely to alter ET-1 release in this preparation as a direct result of flow rate changes. It is therefore likely that the effects of changes in NO synthesis on ET-1 release reported here reflect changes in 'basal' rates of release. ET-1 is known to be a potent vasoconstrictor: these data suggest that inhibition of basal ET-1 release may be a component of the vasodilatory effect of NO.

It is possible that higher NO concentrations in endothelial cells would been achieved by stimulating endogenous production, and this may account for the differences seen. This seems unlikely, however, as sodium nitroprusside was more effective in stimulating cGMP release than was L-arginine. Clearly the inhibition of endothelin release observed in this study was unlikely to be a result of increased cGMP production as L-arginine inhibited both cGMP and ET-1 release, and sodium nitropru-

Table 1
Effect of endogenous NO production on irET-1 release per ml perfusion medium collected.

	irET-1 fmol/ml perfusate	
HBSS	28.2 ± 1.0	$n = 20$
HBSS + 1 mmol/l L-arginine	13.1 ± 0.65***	$n = 22$
TC199	11.5 ± 0.31***	$n = 28$
TC199 + 5 mmol/l L-NAME	28.2 ± 3.0###	$n = 12$

HBSS contains no L-arginine, TC199 contains 230 μmol/l L-arginine and L-NAME is an inhibitor of NO synthase. Values are mean ± S.E.M. ***Indicates value significantly ($P < 0.001$) different from HBSS alone. ###Indicates value significantly ($P < 0.001$) different from TC199 alone. Student's t-test.

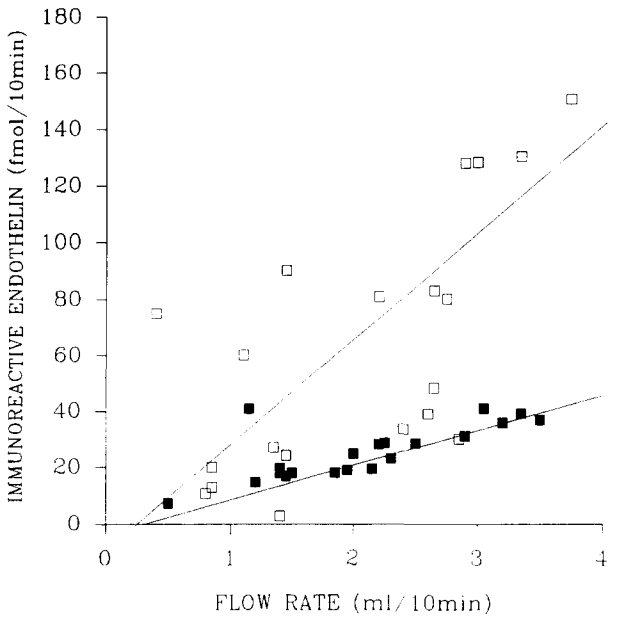

Fig. 4. Effects of perfusing with Tissue Culture Medium 199 in the presence or absence of L-NAME on the correlation between perfusion medium flow rate and irET-1 release by the intact perfused rat adrenal gland. In both cases a highly significant correlation was seen. TC199 alone (■) $r = 0.93$, $P < 0.001$; L-NAME (□) $r = 0.77$, $P < 0.001$.

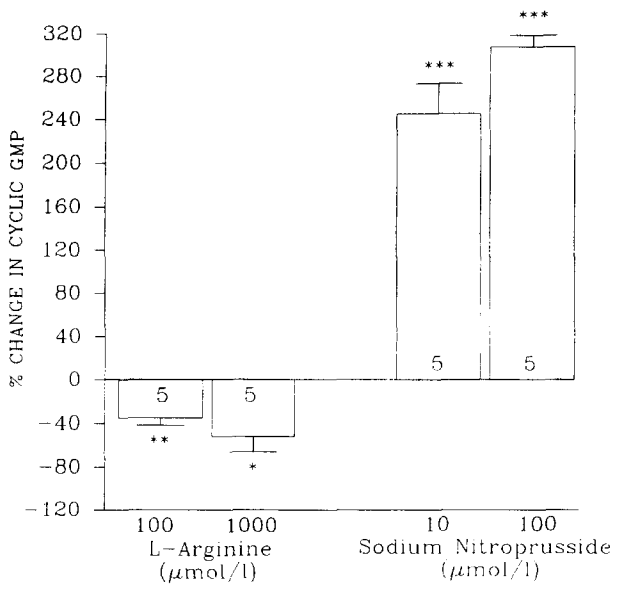

Fig. 5. Effects of adding either L-arginine or sodium nitroprusside (SNP) to HBSS perfusion medium on the release of cyclicGMP from the perfused rat adrenal gland. Values are mean ± S.E.M. Numbers of experiments are indicated in the bars. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared to basal levels obtained with HBSS alone, (analysis of variance).

side, while stimulating cGMP, did not cause inhibition of ET-1 release. Several studies have investigated the effects of altered cGMP production on ET-1 release and have shown that cGMP inhibits ET-1 release. While NO is known to stimulate cGMP production, this action is shared by the natriuretic peptides, which have also been shown to inhibit ET-1 release [19]. An effect of inhibitors of cGMP production on ET-1 release can only therefore be considered as indirect evidence for a role of NO in ET-1 release. It appears that ET-1 release in this perfusion preparation is not controlled by cGMP in the same manner as in the experimental preparations used in other studies.

The question then arises as to the mechanism of the effects reported in the present study: as both L-NAME and L-arginine were used to manipulate endogenous NO production, the effects seen are unlikely to be as a result of a non-specific toxic action of these agents, particularly as D-arginine was without effect. Rather, the effects seen may be due, either to an action of NO which is not mediated by cGMP, or alternatively, another product of NOS activity may be implicated, possibly citrulline. This possibility awaits investigation.

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