Role of endothelins in regulation of vascular tone in the in situ perfused rat adrenals

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Mazzocchi, Giuseppina, Ludwik K. Malendowicz, Francesco G. Musajo, Giuseppe Gottardo, Anna Markowska, and Gastone G. Nussdorfer. Role of endothelins in regulation of vascular tone in the in situ perfused rat adrenals. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E1–E5, 1998.—This study examined the role of endothelins (ETs) and their receptor subtypes ETA and ETB in the regulation of vascular tone in the in situ perfused rat left adrenal gland. Endothelin-1 (ET-1), which binds both ETA and ETB receptors, increased arterial pressure via the ETA receptor, and ETB receptor agonist BQ-3020 magnified the effect of ET-1 and did not affect that of BQ-3020. The ETB-mediated decrease and the ETA-mediated increase in the rate of collection of perfusate were abolished by Ro-31–8220, an inhibitor of protein kinase C (PKC), and by Nω-nitro-L-arginine methyl ester, an inhibitor of nitric oxide synthase (NOS), respectively. Collectively, these findings suggest that ETs can regulate vascular tone in the in situ perfused rat adrenals via both PKC-coupled ETA and NOS-coupled ETB receptors, the activation of which evokes vasoconstriction and vasodilatation, respectively.

Endothelins (ETs) are a family of 21-amino acid peptides mainly secreted by vascular endothelium, which exert a potent and long-lasting vasoconstrictor and pressor activity. The ET family consists of three isopeptides, ET-1, ET-2, and ET-3, which act through two main receptor subtypes, named ETA and ETB, the binding potency of which is ETA, ET-1 > ET-2 >> ET-3; and ETB, ET-1 = ET-2 = ET-3 (12, 22, 25).

Several lines of evidence indicate that ETs and their receptor subtypes are expressed in the adrenal glands, where they appear to exert a paracrine control of steroid secretion and growth (21). In the rat, ETs enhance aldosterone and corticosterone production almost exclusively through the activation of ETB receptors (1, 2, 7) and stimulate proliferation of zona glomerulosa cells via ETB receptors coupled with protein kinase C (PKC) and thyrmosine kinase signaling pathways (18).

The adrenal gland is a highly vascularized organ, with a well-regulated blood supply that is conserved even in the case of severe hemorrhage (3, 27). According to Vinson and Hinson (27), a very close direct correlation between blood flow rate and corticosteroid secretion exists in the rat adrenals, so that any regulatory molecule that is able to modulate adrenal blood flow may indirectly influence steroid secretion. This possibility is likely to occur in the case of several regulatory peptides released by chromaffin medullary cells (20), and findings are available that ET-1 may be included in this group of molecules (6, 13, 14).

The aim of the present study was to examine the role of ETs and their receptor subtypes in the regulation of rat adrenal vascular tone. To this end, we used the technique of the in situ perfusion of the rat left adrenal gland, because it allows for the delivery of the chemical directly to the gland without any possible interference with the systemic mechanism regulating blood pressure and flow. Moreover, in this experimental model adrenal innervation is preserved, which assures the maintenance of a basal vascular tone in the gland (28).

MATERIALS AND METHODS

Animals and reagents. Adult male Wistar rats (260 ± 30 g body weight) were purchased by Charles-River (Como, Italy). ET-1, ET-3, the ETA receptor antagonist BQ-123 (15), the ETB receptor antagonist BQ-788 (16), and the ETB receptor agonist BQ-3020 (20) were obtained from Neosystem Laboratoires (Strasbourg, France). The protein kinase C (PKC) inhibitor Ro-31–8220 (30) was purchased from Roche (Milan, Italy); the nitric oxide (NO) synthase (NOS) inhibitor Nω-nitro-L-arginine methyl ester (l-NAME) (23), adrenocorticotropic hormone (ACTH), and human serum albumin (HSA) were from Sigma Chemical (St. Louis, MO); and Medium 199 was from DIFCO (Detroit, MI).

In situ adrenal perfusion. The rats were anesthetized with pentobarbital sodium, and the left adrenal gland was perfused in situ according to Vinson et al. (28), as previously detailed (18). Perfusion medium was introduced via a cannula inserted in the celiac artery into an isolated segment of aorta from which the adrenal arteries arise; after flowing through the adrenal gland, medium was collected by a cannula inserted in the renal vein. Perfusion medium (tissue culture Medium 199, modified by dilution with KCl-free Krebs-Ringer bicarbonate to give a final K+ concentration of 3.9 mM, and containing 0.2% glucose and 5 mg/ml HSA) was gassed with 95% air-5% CO2, maintained at 37°C, and delivered by a peristaltic pump at a constant rate of 2 ml/10 min for 90 min. Perfusion pressure was monitored by a pressure transducer inserted in the arterial cannula and was found to average 30 ± 3 mmHg. After an initial equilibration period of 30 min, three 10-min samples were collected, and then the perfusion medium was substituted with one in which the chemicals to be tested were dissolved to the required concentration, and three more 10-min samples were collected.

The following chemicals were added to the perfusion medium: 1) ET-1, ET-3, or BQ-3020 (from 10–12 to 10–6 M) alone or in the presence of 10–7 M BQ-123 and/or BQ-788; and 2) 10–8 M ET-1 plus 10–7 M BQ-123 or BQ-788 in the presence or absence of 10–5 M Ro-31–8220 or 10–3 M l-NAME. Some rat adrenals were perfused with the ET receptor antagonists...
and the PKC or NOS inhibitors alone or in the presence of 10^{-9} M ACTH. The concentrations of BQ-123 and BQ-788 were those previously found to completely displace ET-1 binding to ETA and ETB receptors, respectively (2), the concentrations of Ro-31–8220 and L-NAME those completely suppressing PKC activity (18) and endogenous NO synthesis (13), and the concentration of ACTH that is known to raise adrenal blood flow maximally (27, 28).

Perfusion medium flow rate. The volumes of the samples collected before and after the addition of chemicals to the perfusion medium were measured. Not all the delivered medium passes through the adrenal gland and is collected from the renal vein. Some perfusate (~60–80%) is lost throughout the thin arteries arising from adrenal arteries. Because of the great variability of adrenal vessels, this figure varies from rat to rat under basal conditions, but for each rat it exclusively depends on the intraglandular vascular resistance. Hence, the changes in the rate of perfusate collection after the addition of the chemicals are indicative of the variations in the vascular resistance and may provide an estimate of the variations in adrenal blood flow.

Statistics. For each rat, the rate of medium collection was calculated as the average of the three collection periods before and after the injection of the chemicals, and, because of rather elevated variability of this parameter from rat to rat (see above), values were expressed as percent change from baseline. For each experimental point, five rat adrenals were perfused, and the data were expressed as means ± SE. The statistical comparison of the results was done by analysis of variance followed by Duncan's multiple range test. A value of P < 0.05 was considered significant.

RESULTS

ET-1 concentration dependently decreased the adrenal flow rate of the perfusion medium; minimal and maximal effective concentrations were 10^{-10} and 10^{-8} M. The effect of ET-1 was reversed by BQ-123 (10^{-7} M), which evoked a net rise in the rate of perfusate collection. In contrast, BQ-788 (10^{-7} M) magnified the effect of ET-1, which became significant at a ET-1 concentration of 10^{-12} M. When added together, BQ-123 and BQ-788 abolished any effect of ET-1 on the rate of perfusate collection (Fig. 1).

ET-3 evoked a moderate increase in the rate of perfusate flow, which was significant only at a concentration of 10^{-8} M. BQ-123 (10^{-7} M) markedly increased the effect of ET-3, which was already significant at a concentration of 10^{-10} M. BQ-788 (10^{-7} M) reversed the effect of ET-3 and induced a small but significant lowering in the rate of perfusate collection. The simultaneous addition of BQ-123 and BQ-788 annulled the effect of ET-3, the adrenal flow rate of the perfusion medium remaining on the baseline level (Fig. 2). BQ-3020 markedly raised the rate of perfusate flow through the adrenal, the effect being significant at a concentration of 10^{-4} M. BQ-788 abolished the effect of BQ-3020, whereas BQ-123 was ineffective (Fig. 3).

In the presence of 10^{-7} M BQ-788, ET-1 decreased the adrenal perfusate flow rate, and 10^{-5} M Ro-31–8220 abolished this effect of ET-1, whereas 10^{-3} M L-NAME was ineffective (Fig. 4). Conversely, in the presence of 10^{-7} M BQ-123, ET-1 increased the rate of perfusate collection, and 10^{-3} M L-NAME blocked this effect, whereas 10^{-5} M Ro-31–8220 did not affect it (Fig. 5).

The administration of BQ-123, BQ-788, Ro-31–8220, or L-NAME alone did not evoke any significant change in the rate of perfusate collection, nor did these inhibitors affect the well-known adrenal vasodilatory action of ACTH (27) (Table 1).

DISCUSSION

Our present findings, although obtained by an experimental approach that surely does not exactly reproduce physiological conditions, confirm the view that ETs play a role in the regulation of adrenal blood flow in the rat through a mechanism involving the modulation of intraglandular vascular resistances (21).

Compelling evidence indicates that ET-1 binds to both ETA and ETB receptor subtypes, whereas ET-3 preferentially binds to the ETB receptor (12, 22, 25). Hence, the effects of ET-1 on vascular resistance in adrenals are the results of the simultaneous activation of ETA and ETB receptors, whereas those of ET-3 are mainly the expression of the ETB receptor activation. ET-1 and ET-3 exert opposite effects on intra-adrenal vascular resistances, the former increasing and the latter peptide decreasing them. The ETA receptor selective antagonist BQ-123 reverses ET-1 effect and enhances ET-3 effect, whereas the opposite is true for the ETB receptor antagonist BQ-788. The selective ETB receptor agonist BQ-3020 exerts a more potent ET-3-like effect, which is abrogated by BQ-788. Collectively, these results allow us to suggest that 1) the activation of ETA receptors raises and that of ETB receptors lowers intra-adrenal vascular resistances and that, 2) under...
our experimental conditions, the ET-1-induced activation of ETA receptors overcomes that of ETB receptors.

The first hypothesis appears to be in keeping with the general contention that the isopeptide-selective ETA receptor is mainly expressed in vascular smooth muscle cells and mediates vasoconstriction through phospholipase C-dependent pathways, whereas the nonisopeptide-selective ETB receptor is mainly expressed in endothelial cells and is involved in the endothelium-dependent vasodilation mediated by NO release (25, 26). Accordingly, our results show that the reduction

Fig. 2. Effects of ET-3, in the presence or absence of 10^{-7} M ET receptor antagonists, on flow rate of perfusion medium in situ perfused rat adrenal glands. Values are percentage changes (means ± SE; n = 5). Baseline value (B) 0.65 ± 0.03 ml/10 min. Significant differences: *P < 0.05 and **P < 0.01 vs. baseline; #P < 0.05 and $P < 0.01 vs. respective control value.

Fig. 3. Effects of BQ-3020, in the presence or absence of 10^{-7} M ET receptor antagonists, on flow rate of perfusion medium in situ perfused rat adrenal glands. Values are percentage changes (means ± SE; n = 5). Baseline value (B) 0.70 ± 0.04 ml/10 min. **P < 0.01 vs. baseline; $P < 0.01 vs. respective control value.

Fig. 4. Effects of 10^{-5} M Ro-31–8220 and 10^{-3} M L-NAME on decrease in perfusion medium flow rate induced by activation of ETA receptors (increasing concentrations of ET-1 in presence of 10^{-7} M BQ-788). Values are percentage changes (means ± SE; n = 5). Baseline value (B) was 0.62 ± 0.04 ml/10 min. *P < 0.05 and **P < 0.01 vs. baseline; #P < 0.05 and $P < 0.01 vs. respective control value.

Fig. 5. Effects of 10^{-5} M Ro-31–8220 and 10^{-3} M N^{G}-nitro-L-arginine methyl ester (L-NAME) on increase in perfusion medium flow rate induced by activation of ETB receptors (increasing concentrations of ET-1 in the presence of 10^{-7} M BQ-123). Values are percentage changes (means ± SE; n = 5). Baseline value (B) was 0.58 ± 0.03 ml/10 min. **P < 0.01 vs. baseline; #P < 0.01 vs. respective control value.
ETB receptor subtypes are expressed in the rat adrenal (11, 22). It remains to be settled whether both vasodilatory effect of ETB1 appears to mask the vasoconstriction of ET-3 or BQ-3020. Anyway, it must be recalled that the administration being a net increase in adrenal vascular resistance overcomes that of ETB receptors, the net effect of ET-1 plus the selective antagonist of ETB receptors Ro-31–8220 and the NOS inhibitor L-NAME, respectively. Parenthetically, because of the present unavailability of specific ETB agonists, the exclusive activation of this receptor subtype was obtained by administering ET-1 plus the selective antagonist of ETB receptors BQ-788. Recent investigations have distinguished two subtypes of ETB receptors, ETB1 and ETB2, based on their affinity to the ETB selective ligand RES-701–1 (22). Both receptors are located in the smooth-muscle cells of systemic and kidney vessels; the RES-701–1-sensitive ETB1 would mediate vasodilation, and the RES-701–1-insensitive ETB2 would mediate vasoconstriction (11, 22). It remains to be settled whether both ETB receptor subtypes are expressed in the rat adrenal vasculature and to what extent ETB2 activation may contribute to the effect of ET-1 and counteract that of ET-3 or BQ-3020. Anyway, it must be recalled that the vasodilatory effect of ETB1 appears to mask the vasoconstrictor effect of ETB2 in the systemic and kidney vessels of rats (11).

According to Hinson and co-workers (13, 14), our findings indicate that, in the in situ adrenal perfusion model, the ET-1-induced activation of ETB receptors overcomes that of ETA receptors, the net effect of ET-1 administration being a net increase in adrenal vascular tone. This observation seems to conflict with the conclusions of Gellai et al. (11) that, in vivo, the predominant role of endogenous ETs is vasodilation and that ETA receptors play a negligible role in the control of vascular tone in the rat. These discrepancies may be easily reconciled by considering that, in our model, exogenous ETs are delivered to the gland periphery by adrenal arteries, and by taking into account the differential adrenal distribution of ETA and ETB receptors, which in turn reflects the peculiar vascular arrangement of the gland.

The main adrenal arteries divide near the gland capsule, forming a plexus from which arterioles arise that penetrate into the outer portion of zona glomerulosa, where they open in the capillary network of the cortex; this last drains into the medullary veins, which in turn give rise to the central vein that opens into the renal vein. At variance with humans and dogs (3, 27), in the rat only exceptionally do arterioles arising from capillary plexus pass throughout the cortex, directly reaching the medulla (27), thereby making unlikely the possibility of a relative independence of the cortical and medullary blood flows in this species. The central adrenomedullary vein does not behave as a passive capacitance vessel but contributes to the regulation of the venous outflow from the gland, its wall provided with a relatively thick tunica muscularis that can contract (10, 17, 19, 29). Hence, only the subcapsular portion of the cortex and the medulla contain a sizable amount of vascular smooth-muscle cells, which explains why autoradiographic studies of the selective displacement of 125I-labeled ET-1 binding show evidence of ETA receptors almost exclusively located in the zona glomerulosa and adrenal medulla (1, 2, 8, 24). The “strategic” position of the ETA receptor may explain why the first effect of ETs delivered to the adrenal gland may be the vasoconstriction of subcapsular arterioles, with the ensuing decrease in the flow rate of the perfusion medium, which cannot be efficaciously counteracted by the subsequent activation of ETB receptors, mainly located in the endothelial lining of the capillary network. Another important target of ETs may be the central adrenomedullary vein, whose contraction provokes a further decrease in the rate of perfusate recovery. This last possibility receives support by the demonstration that ETs, via ETA receptors, induce contraction of isolated segments of the bovine central medullary vein (17).

The finding that neither BQ-123 and BQ-788 nor Ro-31–8220 and L-NAME are per se able to affect intra-adrenal vascular resistances deserves some discussion, inasmuch as they appear to rule out the possibility that endogenous ETs may be involved in the physiological regulation of adrenal blood flow. Moreover, they do not agree with the results of Hinson and co-workers (13, 14), who observed that BQ-123 (5 × 10−7 M) evokes a 20% rise in the perfusion medium flow rate in adrenals and that L-NAME (5 × 10−3 M) induces a 60% decrease when medium contains the substrate for NO synthesis. In addition, earlier investigations using a radiolabeled microsphere methodology have consistently shown that L-NAME exerts per se a marked constrictive action on dog adrenal vasculature (3, 4). In light of these findings, investigators advanced the hypothesis that endogenous ETs and NO exert tonic vasoconstrictor and vasodilatory actions, respectively, on adrenal vasculature, a contention in conflict with the results of Gellai et al. (11) as far as ET-1 and ETA are concerned.

Only hypotheses may be advanced at present to reconcile the discrepancies between our present findings and those of Hinson and co-workers (13, 14), because both were obtained using the same experimental model of in situ perfusion of rat adrenals. Although shear stress seems to inhibit ET synthesis in vascular endothelium (25), evidence indicates that a moderate shear stress, like that generated by an increase in the blood flow rate, stimulates the release of these peptides (9). Accordingly, Cameron et al. (6) demonstrated that a close direct correlation exists between the flow rate of...
the perfusion medium and ET-1 concentration in the venous effluent in in situ perfused rat adrenals; this finding was obtained by mechanically increasing the rate of perfusion from a baseline value of 6 ml/10 min, which is three times higher than that used in our present experiments, to a maximum of 20 ml/10 min. Preliminary radioimmunoassay of ET-1 immunoreactivity in the perfusate collected in our experiments gave negative results (sensitivity of our assay, 8 fmol/ml). Hence, it does not seem unreasonable to conceive that, in this experimental model, the tonic activation of vascular ET receptors by locally released endogenous ET-1 has to be considered an artifact caused by the high rate of adrenal in situ perfusion, probably deprived of any physiological relevance under basal condition of adrenal blood flow.

To summarize, our present study allows us to draw the following conclusions: 1) exogenous ETs regulate adrenal vascular tone in the rat via both ETA and ETB receptor subtypes; 2) the activation of ETA receptors evokes vasoconstriction and that of the ETB receptor evokes vasodilation through a PKC-dependent and a NOS-dependent signaling pathway, respectively; and, finally, 3) the involvement of locally released ETs in the physiological maintenance of rat adrenal blood flow under basal conditions is doubtful and remains to be investigated.

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REFERENCES


