EFFECTS OF THE PROLONGED ADMINISTRATION OF SELECTIVE ETₐ- AND ETₐ-ReCEPTOR ANTAGONISTS ON THE RAT ADRENAL CORTEX

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ABSTRACT

The role of endogenous endothelins (ETs) in the functional regulation of the pituitary-adrenal axis (PAA) and in the maintenance of rat adrenal growth has been investigated. The daily subcutaneous injection of the selective ETₐ- and ETₐ-receptor antagonists, BQ-123 and BQ-788 (20 nmol/kg for 5 days), did not evoke any significant changes in the adrenal weight, as well as in the growth of adrenocortical zones and cells, but it caused significant rises in the plasma concentrations of ACTH, aldosterone and corticosterone. However, the prolonged administration of the two antagonists inhibited the acute secretory responses of PAA to ET-1 (1.5 nmol/kg). In light of these findings, the following conclusions are drawn: (i) endogenous ETs do not play a major role in the physiological maintenance of rat adrenal growth; (ii) exogenous ET-1 acutely stimulates rat PAA axis acting through both BQ-123- and BQ-788-sensitive ET receptors (ETₐ and ETₐ); and (iii) the occupancy of ETₐ and ETₐ by the two ligands, probably allows endogenous ETs to bind molecular variants of ET receptors (ETₐ and ETₐ) whose prolonged activation is able to stimulate rat PAA.

Endothelins (ETs) and their receptor subtypes A and B (ETₐ and ETₐ) are expressed in the rat hypothalamo-pituitary-adrenal axis (for review, see 7, 9, 10). We have recently demonstrated that ET-1 stimulates in vivo pituitary ACTH release and adrenocortical secretion in rats, acting through both ETₐ and ETₐ receptors, inasmuch as ET-1 effects are counteracted by BQ-123 and BQ-788, which are selective antagonists of ETₐ- and ETₐ-receptor subtypes, respectively (3, 4). However, the two antagonists, when administered alone, were ineffective, thereby casting doubts about the physiological relevance of the role of endogenous ETs in the acute regulation of pituitary and adrenal functions, at least under basal conditions.

Compelling evidence indicates that ETs also exert a long-term stimulatory effect on the growth and secretory capacity of the rat adrenal cortex (5, 6), and the aim of the present investigation was to ascertain whether the prolonged suppression of ETₐ- and ETₐ-receptor activation by endogenous ETs is able to alter pituitary and adrenal functions.

MATERIALS AND METHODS

Adult female Wistar rats (190–200 g body weight) were kept under a 12:12 h light-dark cycle (illumination onset at 8.00 a.m.) at 23°C, and maintained on a standard diet and tap water ad libitum. ET-1 (human-porcine sequence), and the ETₐ and ETₐ selective antagonists BQ-123 and BQ-788 (8) were purchased from Neosystem Labs (Strasbourg, France). ACTH-RIA kit was obtained from Diagnostic Products (Los Angeles, CA, U.S.A.).

The rats were divided into equal groups (n = 16), which were given at 9.00 a.m. daily subcutaneous injections of BQ-123 or BQ-788 (20 nmol/
kg) dissolved in 0.2 ml 0.9% NaCl (vehicle) for 5 days. A third group was injected with the vehicle only (control group). On day 6, half of the animals in each group was given at 9:00 a.m. a subcutaneous injection of ET-1 (1.5 nmol/kg) dissolved in 0.2 ml vehicle, and the other half was injected with the vehicle only. The rats were decapitated 60 min after the last injection. The trunk blood was collected in the presence of EDTA, and plasma was separated and stored at −30°C. Adrenal glands were promptly removed and freed of adherent fat; their weight was recorded.

Adrenal glands were fixed in Bouin’s solution, embedded in paraffin, and serially cut at 5–6 μm of thickness. Sections were stained with hematoxylin-eosin, and the volume of zona glomerulosa, zona fasciculata and zona reticularis, as well as the average volume and number of their parenchymal cells were calculated according to Weibel (11), as previously detailed (1).

ACTH, aldosterone and corticosterone were extracted from plasma, and their concentrations were assayed by RIA, as described earlier (2). Intra- and interassay variation coefficients were: ACTH, 6% and 8%; aldosterone, 5% and 7%; and corticosterone, 7% and 9%, respectively.

Individual results were averaged per experimental group, and SE (n = 8) was calculated. The statistical comparison of the data was done by ANOVA, followed by the Multiple Range Test of Duncan.

RESULTS AND DISCUSSION

Neither BQ-123 nor BQ-788 administered for 5 consecutive days evoked significant changes in the body and adrenal weights (Fig. 1). Likewise, morphometric parameters of the adrenal cortex did not display significant variations, with the exception of the average volume of zona fasciculata cells, which underwent a 20% decrease after BQ-123 treatment (Fig. 2).

These findings appear to rule out the possibility that endogenous ETs, acting through the activation of ET_A and ET_B receptors, play a major role in the maintenance of adrenal growth in the rat. At present, we are not able to explain the BQ-123-induced slight atrophy of zona fasciculata cells, if not hypothesizing that this antagonist exerts a toxic effect specifically addressed against the mechanisms involved in the maintenance and stimulation of the growth of this adrenocortical-cell type.

Both BQ-123 and BQ-788 treatment markedly raised basal plasma concentrations of ACTH (3.0-fold and 3.5-fold), aldosterone (75% and 73%) and corticosterone (2.1-fold and 2.3-fold) (Fig. 3), thereby suggesting that the prolonged inactivation of ET_A and ET_B receptors is able to stimulate rat pituitary-adrenal axis (PPA). In contrast, BQ-123 treatment partially inhibited (from 30% to 40%) and BQ-788 treatment completely reversed ACTH, aldosterone and corticosterone acute responses to ET-1 (Fig. 4), a finding confirming the view that exogenous ETs activate rat PPA acting through both ET_A and ET_B receptors (3, 4).

These apparently conflicting results are intriguing but very difficult to explain. However, molecular variants of ET_A and ET_B receptors insensitive to BQ-123 and BQ-788, respectively, have recently been described. ET_A1 and ET_B1 are sensitive to BQ-123 and BQ-788, while ET_A2 and ET_B2 are not, and have been reported to mediate some vascular ET_A or ET_B-mediated responses to ETs which are not suppressible by BQ-123 or BQ-788 (for review, see 8). It could be assumed that these ET-receptor variants are present in the rat PAA and play a relevant role in its activation, and the observation that BQ-123 and BQ-788, when administered alone, elicit a clear-cut stimulation of the PAA accords well with this contention. In fact, the occupancy of the ET_A1 and ET_B1 receptor subtypes by BQ-123 and BQ-788 might increase the binding of endogenous ETs to the molecular variants coupled with PAA activation. This hypothesis obviously entails...
that exogenously administered ET-1 exerts its PAA stimulating action acting exclusively via the classic BQ-123- and BQ-788-sensitive ET<sub>A</sub> and ET<sub>B</sub> receptor subtypes.

Further studies employing selective antagonists of ET<sub>A</sub> and ET<sub>B</sub> subtypes (e.g. PD 142993 and SB 209670, respectively) are under way in our laboratory to settle the possible physiological role of endogenous ETs in the functional regulation of rat PPA.
Fig. 3  Effects of the prolonged administration of the ET-receptor antagonists on the plasma concentration of ACTH, aldosterone and corticosterone. Bars are means ± SE (n = 8). A, controls; B, BQ-123; C, BQ-788. *P < 0.01 from A

Fig. 4  Effects of the prolonged administration of the ET-receptor antagonists on the acute response of plasma concentrations of ACTH, aldosterone and corticosterone to ET-1. Bars are means of % changes from baseline ± SE (n = 8). A, controls; B, BQ-123; C, BQ-788. *P < 0.05 and ^P < 0.01 from A

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