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Short communication

Endothelin-3 can both facilitate and inhibit transmitter release in the guinea-pig vas deferens

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Abstract

Endothelin-3 (10 nM) produced a significant facilitation of the release of ATP from the in vitro guinea-pig vas deferens. This effect was converted to an inhibition of release by pretreatment with BQ-123, cyclo-(D-Trp,D-Asp,L-Pro,D-Val,L-Leu), an endothelin ET_A receptor antagonist. Desensitization of endothelin ET_B receptors by sarafotoxin S6c antagonized, but did not reverse, the facilitatory effect of endothelin-3. The release of noradrenaline was not facilitated by endothelin-3; however, following pretreatment with BQ-123 the release of noradrenaline was reduced by the peptide. These results indicate that there may be both facilitatory and inhibitory prejunctional endothelin receptors and further suggest that the release of the sympathetic nerve cotransmitters ATP and noradrenaline may be differentially modulated.

Keywords: Endothelin receptor; Cotransmission; Vas deferens; Endothelin-3; BQ-123; Sarafotoxin S6c; (Guinea-pig)

1. Introduction

The endogenous peptides of the endothelin family exhibit a variety of biological effects including potent actions at autonomic neuroeffector junctions (Wiklund et al., 1991; Warner et al., 1993; Mutafova-Yambolieva and Radomirov, 1993, 1994). Sometimes it is difficult to determine whether the actions of the endothelins at the neuroeffector junction are primarily prejunctional, postjunctional or both. For example, endothelin-3 has been reported to enhance the nerve induced phasic contraction of the rodent vas deferens (Wiklund et al., 1991; Warner et al., 1993) but is without effect on the evoked release of [^{3}H]noradrenaline. These results imply that the effect of endothelin-3, in this situation, may be entirely postjunctional, that is, endothelin-3 potentiates the action of the released transmitter at a postjunctional site rather than influencing the release of transmitter. However, neurotransmission to the smooth muscle of the vas deferens involves at least two cotransmitters, ATP and noradrenaline (Sneddon and Westfall, 1984). Furthermore, the initial phasic contraction of the vas deferens to nerve stimulation is mediated mainly by ATP with noradrenaline making a smaller contribution. Thus, even though endothelin-3 does not apparently increase the nerve evoked release of noradrenaline, it may influence the release of ATP.

While it is generally believed that alteration in the release of one transmitter should do likewise to the cotransmitter, recently evidence has emerged indicating that the release of ATP and noradrenaline may be differentially influenced (Burnstock, 1990; Todorov et al., 1994). In the present study we have examined the influence of endothelin-3 and putative ET receptor antagonists on the electrical field stimulation-induced release of endogenous noradrenaline and ATP in the guinea-pig vas deferens.

2. Materials and methods

Vasa deferentia from adult guinea-pigs were superfused with a physiological salt solution (Sedaa et al., 1990) at 35°C in a 200 μl chamber that had platinum screen electrodes at each end. After equilibration, the tissues were subjected to electrical field stimulation (EFS) with 0.1 ms square wave pulses at 8 Hz for 1 min. Samples of the superfusion solution were collected before and during the EFS and analyzed for the

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content of noradrenaline, by high-performance liquid chromatography (HPLC) with electrochemical detection, and ATP, by HPLC-fluorescence detection (Sedaa et al., 1990). Endothelin-3 was added 20 min prior to EFS. In some experiments, the effect of endothelin-3 was examined after pretreating tissues for 20 min with the endothelin ET_A receptor antagonist cyclo-(D-Trp,D-Asp,L-Pro,D-Val,L-Leu) (BQ-123). In other experiments the tissues were treated for 45 min with sarafotoxin S6c to desensitize the endothelin ET_B receptors.

Endothelin-3 was obtained from Sigma Chemical Company (St. Louis, MO, USA) and BQ-123 and sarafotoxin S6c from Peninsula Laboratories (Belmont, CA, USA). Data were analyzed statistically by two-tailed Student's *t*-test for unpaired data. A P value of less than 0.05 was considered significant.

3. Results

Endothelin-3 (10 nM) did not on its own cause release of ATP or noradrenaline (data not shown) but did produce a substantial increase in the electrically evoked release of ATP (Fig. 1a). Electrical field stimulation-evoked release of noradrenaline was not enhanced by endothelin-3 (Fig. 1b). Pretreatment with BQ-123 (0.5 μM) alone did not significantly change the electrically evoked release of either ATP or noradrenaline. However, pretreatment with BQ-123 completely prevented the facilitatory effect of endothelin-3 on evoked release of ATP and in fact BQ-123 reversed the action of endothelin-3, i.e., after treatment with BQ-123, endothelin-3 caused a significant inhibition of electrically evoked release of ATP. Interestingly, endothelin-3, which was without a significant effect on electrical field stimulation-evoked release of noradrenaline, caused a significant decrease in evoked release of noradrenaline after pretreatment with BQ-123. Prolonged pretreatment with sarafotoxin S6c (100 nM), which is known to first stimulate endothelin ET_B receptors and then to desensitize the endothelin ET_B receptors to subsequent stimulation by agonists, prevented the facilitatory effect of endothelin-3 on electrically evoked release of ATP but unlike BQ-123, did not reverse the action of endothelin-3.

4. Discussion

Endothelin-3 has been shown to increase the magnitude of the neurogenically induced phasic contraction of the rodent vas deferens (Wiklund et al., 1991; Warner et al., 1993). The phasic contraction is due primarily to the action of ATP released as a cotransmitter from the sympathetic nerves (Sneddon and Westfall, 1984). The enhancement of the phasic response of the vas deferens by endothelin-3 could be due to a potentiation of the postjunctional action of ATP or to an enhancement of the evoked release of ATP from the sympathetic nerves. To our knowledge neither the effect of endothelin-3 on the contractile response of the vas deferens to exogenously applied ATP nor its effect on electrical field stimulation-evoked release of ATP has been investigated. However, endothelin-1 enhances the response to exogenous ATP in the guinea pig and rat vas deferens (Wiklund et al.,

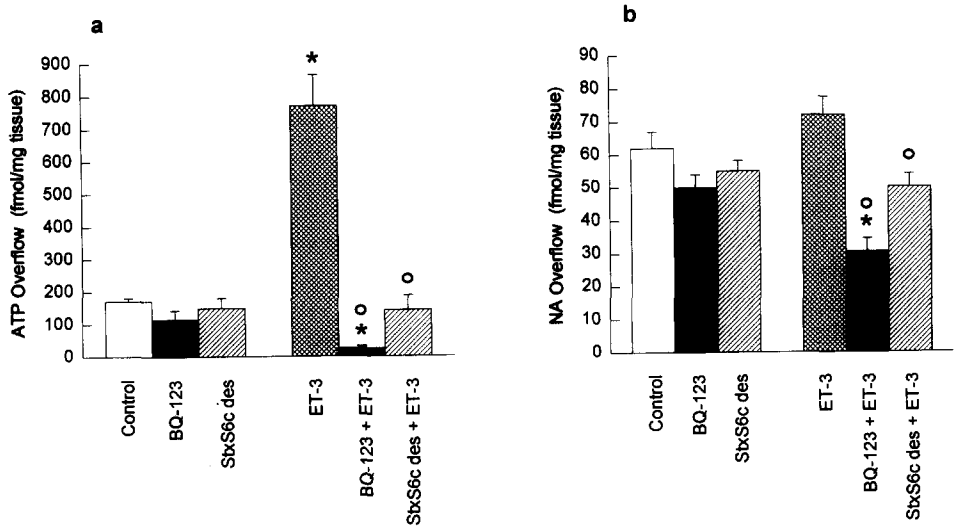

Fig. 1. The effect of endothelin-3 (ET-3) and antagonists on the EFS-evoked overflow of ATP (panel a) and noradrenaline (panel b) from sympathetic nerves of the guinea pig vas deferens. Overflow was evoked by 0.1 ms pulses at 8 Hz for 1 min. Each column shows the mean and S.E.M. of 4–13 experiments. The asterisk indicates a significant difference from control and the circle indicates a significant difference from endothelin alone.

1991; Mutafova-Yambolieva and Radomirov, 1993) and the rabbit saphenous artery (Mutafova-Yambolieva and Radomirov, 1994). Thus it is possible that endothelin-3 does the same.

In addition to a possible postjunctional action, we reasoned that endothelin-3 may also enhance the release of ATP. There is precedence for the idea that endothelins may increase the release of transmitters. For example, in studies with the isolated rabbit bronchus and rat iris sphincter, endothelin-3 potentiated cholinergic nerve induced contractions without affecting the postjunctional sensitivity to cholinergic agonists. Therefore it was concluded that endothelin-3 enhanced transmitter release (McKay et al., 1993; Shinkai et al., 1994). This issue is far from clear, however, because Wiklund et al. (1991) reported that endothelin-3 reduced the evoked release of[^{3}H]acetylcholine from parasympathetic nerves of the guinea-pig ileum.

In the current study we examined the effect of endothelin-3 on the EFS-evoked release of endogenous ATP as well as the cotransmitter noradrenaline. Endothelin-3 produced a substantial increase in the evoked release of ATP, an effect that quite likely contributes to the increased magnitude of the phasic contractile response of the vas deferens.

An interesting feature of our results is that endothelin-3 enhanced the evoked release of ATP without significantly enhancing the release of the cotransmitter noradrenaline. Wiklund et al. (1991) also reported that endothelin-3 was without effect on the release of [^{3}H]noradrenaline in rat vas deferens. One possible explanation for this differential effect on the cotransmitters would be if the release of ATP occurred from extraneuronal sites as well as from the sympathetic nerves while noradrenaline release occurs only from the nerves with endothelin-3 enhancing release only from extraneuronal sites. In some in vitro neuroeffector preparations there is evidence for release of ATP from an extraneuronal source as well as from a neuronal source. In blood vessels, for example, α_1-adrenoceptor activation leads to ATP overflow from the endothelium (Sedaa et al., 1990). However, an extraneuronal source of ATP does not seem to be involved in our experiments with vas deferens. Previous work in our laboratory by Todorov et al. (1994), using procedures virtually identical to those employed in the present study, indicate that neither postjunctional α_1-adrenoceptor activation nor contraction of the smooth muscle per se cause ATP release. Additional unpublished data from our laboratory also indicate that the overflow of ATP originates from a neuronal source and is independent of an action of noradrenaline. For example, the overflow of ATP is not reduced by depletion of noradrenaline by pretreatment with reserpine nor by blocking α_1-adrenoceptors with prazosin. Guanethidine, an agent known to disrupt the action potential induced release of transmitters from adrenergic nerves, abolished the release of ATP and noradrenaline. We conclude therefore that ATP overflow measured in these studies reflects primarily release from sympathetic nerves. Thus, the differential effect of endothelin-3 on the release of ATP and noradrenaline appears to be another example of the differential regulation of the release of cotransmitters (Burnstock, 1990) and is consistent with the idea that the two cotransmitters may be released from two populations of exocytotic vesicles in this tissue, one of which releases predominantly ATP and the other predominantly noradrenaline (Todorov et al., 1994).

The facilitatory effect of endothelin-3 on the release of ATP was antagonized by BQ-123, a known antagonist of endothelin ET_A receptors. This finding would indicate the involvement of endothelin ET_A receptors in this phenomenon. However, sarafotoxin S6c, an agent reported to be selective for endothelin ET_B receptors (Masaki et al., 1994) also effectively blocked the facilitatory action of endothelin-3. Thus, the receptors that mediate the facilitatory actions of endothelin-3 on ATP release have some features of an endothelin ET_A receptor and some of an endothelin ET_B receptor. This may mean that both endothelin ET_A and ET_B receptors contribute to the facilitation of ATP release by endothelin-3, or that an unusual, as yet unidentified, receptor mediates this action. Recent information from molecular genetics studies indicates that there is a third type of endothelin receptor, the endothelin ET_C receptor, which has a high affinity for endothelin-3 (Karne et al., 1993). Whether such a receptor, which thus far has only been identified in non-mammalian tissues (Masaki et al., 1994) is involved in the prejunctional actions of endothelin-3 requires further study. It is interesting to note that based on studies of the mechanical response of several smooth muscles, including the vas deferens, an atypical receptor, specific for endothelin-3, has been proposed previously (Eglezos et al., 1993; Battistini et al., 1994).

After antagonism of the facilitatory action of endothelin-3 by BQ-123, endothelin-3 caused a significant decrease in the electrically evoked release of both ATP and noradrenaline. This suggests that there may be both facilitatory and inhibitory prejunctional actions, and perhaps both facilitatory and inhibitory prejunctional endothelin receptors, on the sympathetic nerves of the vas deferens.

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