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The vascular relaxant effects of guanabenz are not mediated by α_2-adrenoceptors in rat aortic rings

P. SOARES-DA-SILVA, M. M. VILLANUEVA*, *Laboratório de Farmacologia, Faculdade de Medicina, 4200 Porto, Portugal*

Abstract—The present experiments show that the α_2-adrenoceptor agonist guanabenz produced a concentration-dependent relaxation in 5-hydroxytryptamine- and noradrenaline-preconstricted rat aortic rings, but not when tone was raised by high potassium in the medium. Similar findings were made for pinacidil. In 5-hydroxytryptamine-preconstricted preparations the relaxant effect of guanabenz was not affected by yohimbine, propranolol or by removal of the endothelium. In noradrenaline-preconstricted rings the relaxant effects of guanabenz and pinacidil were significantly antagonized by tetraethylammonium. The results suggest that guanabenz might act in the muscle tissue by opening potassium channels or antagonism of calcium mobilization from intracellular stores.

Guanabenz and guanfacine, two guanidine derivatives, are potent antihypertensive drugs and like clonidine, believed to act as central α_2-adrenoceptor agonists. However, when given acutely by the intravenous route all three compounds produce a pressor response which has been ascribed to the activation of postjunctional α-adrenoceptors in vascular smooth muscle cells (Kobinger 1978). Unexpectedly, we recently found in dogs that the pressor responses to these three α_2-adrenoceptor agonists were not of the same magnitude, guanabenz having a much weaker pressor effect. Also, in preconstricted dog isolated saphenous vein rings, a preparation known to have a considerable population of postjunctional α_2-adrenoceptors (De Mey & Vanhoutte 1981), guanabenz alone elicited concentration-dependent relaxant response, an effect not blocked by yohimbine or propranolol (unpublished data). We now describe our findings on the vascular smooth muscle relaxing properties of guanabenz in the rat thoracic aorta, a preparation in which α_2-adrenoceptors constitute a less important subpopulation of receptors (Descombes & Stoclet 1985), with the aim to further characterize the nature of the mechanisms involved.

Materials and methods

Male Wistar rats, 200 g, were decapitated, exsanguinated and the thoracic aorta excised, trimmed of fat and connective tissue and four rings cut. In some rings endothelium was removed by gentle rubbing of the intimal surface. Intact rings and rings with disrupted endothelium were suspended in a 10 mL organ bath of Krebs solution at 37°C, gassed with 95% O_2 plus 5% CO_2. The Krebs solution had the following composition (mM): Na^+ 118, K^+ 4·7, $CaCl_2$ 2·4, KH_2PO_4 1·4, $MgSO_4$ 1·2, $NaHCO_3$ 25, glucose 11; ascorbic acid 30 μM, disodium edetate (EDTA) 11·4 μM was also added. During a 60–90 min equilibration period the rings were stretched to a final resting tension of 1·0 g, the Krebs solution being changed every 20 min. Contractions were measured isometrically by means of a Gould Statham force transducer (model UC3) and recorded on a Letica model 4000 polygraph. To display relaxant responses aortic rings were preconstricted with either noradrenaline (100–300 nM) 5-hydroxytryptamine (5-HT) (2μM) or potassium chloride (20–30 mM); this resulted in 60–70% of the maximal contraction. On this background of tone the responses to cumulative concentrations of guanabenz (Sandoz, Nurnberg, FRG) and pinacidil (pinacidil monohydrate; Leo Pharmaceuticals, Ballerup, DK) were recorded, results being expressed as mean ± s.e.m. The functional integrity of endothelium in unrubbed rings was confirmed by the relaxing response to carbachol (Sigma, St. Louis, Mo, USA). When the effects of yohimbine (1 μM) (Sigma), propranolol (1 μM) (Sigma) or tetraethylammonium (L. Light & Co. Ltd, Colnbrook, UK) (10 mM) on the responses to guanabenz and pinacidil were studied the tissues were exposed to the drug for 30 min, then reconstricted with either 5-HT or noradrenaline and cumulative concentration-dependent curves to the relaxants repeated.

Correspondence to: P. Soares-da-Silva, Laboratório de Farmacologia, Faculdade de Medicina, 4200 Porto, Portugal.
*On leave from: Dept. de Fisiologia y Farmacologia, Universidad de Alcala de Henares, España.

Results

The addition of increasing concentrations of guanabenz to 5-HT-preconstricted rat thoracic aorta rings produced a concentration-dependent relaxation (Fig. 1a). As also shown, removal of the endothelium did not modify the drug's relaxant effect; the absence of a functional active endothelium was checked by the addition of carbachol which did not produce any relaxation. Also, the relaxant effects of guanabenz did not differ in control or yohimbine- (1 μM) treated preparations (Fig. 1a).

When rat thoracic aortic rings were constricted with noradrenaline, guanabenz still produced a concentration-dependent relaxation (Fig. 1b). However, when tone was raised with high potassium (20–30 mM) this was abolished. With pinacidil, too, the relaxant effects could be only elicited when preparations had been constricted with noradrenaline, but not with high potassium (Fig. 1c). The dose-response curves of the relaxant effects of guanabenz and pinacidil against noradrenaline-induced constriction were shifted to the right in the presence of tetraethylammonium (10 mM).

Discussion

The results clearly show that the α_2-adrenoceptor agonist guanabenz consistently produces a concentration-dependent relaxing effect. Several authors (Egleme et al 1984; Miller et al 1984), using similar experimental conditions have shown that the constrictor effect elicited through activation of postjunctional α_2-adrenoceptors in rat aortic rings denuded of endothelium was greater than that observed in preparations with the endothelium intact. However, as shown in Fig. 1a, the relaxant effect of guanabenz was of the same magnitude on preparations with and without endothelium. The drug also relaxes preparations exposed to the α_2-adrenoceptor antagonist yohimbine, which further suggests that guanabenz is not relaxing rat aortic preparations through activation of α_2-adrenoceptors. The relaxant effect also cannot be attributed to the activation of β-adrenoceptors since propranolol did not reduce its relaxant effects.

Although it could be argued that the relaxant effect of guanabenz might be due to an unexpected antagonistic action upon 5-HT receptors, this appears not to be the case, since guanabenz also relaxed noradrenaline-preconstricted preparations with a similar profile (Fig. 1b). In contrast, aortic rings

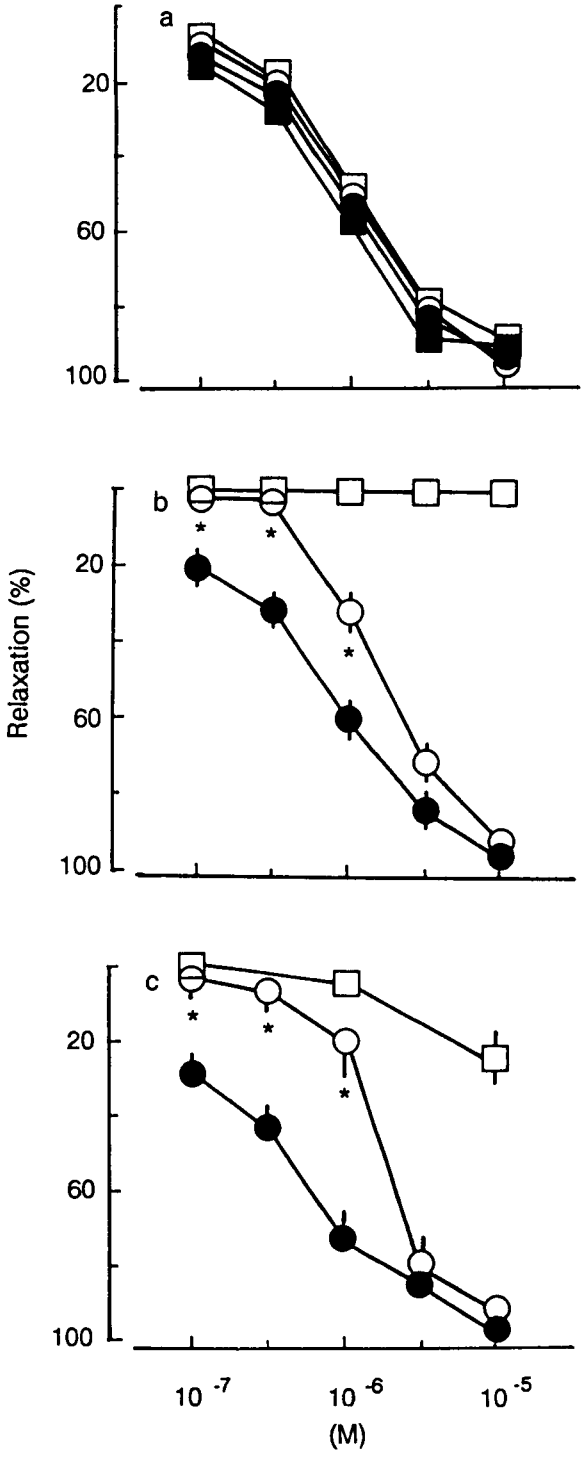

FIG. 1. (a) Effect of guanabenz on rat thoracic aortic rings (●) with and (○) without endothelium and in the presence of (■) yohimbine (1 μM) or (□) propranolol (1 μM), during contraction due to 5-hydroxytryptamine (2 μM). (b) Effect of guanabenz on rings during contraction to (●) noradrenaline (100 nM), (○) noradrenaline (100 nM) in the presence of tetraethylammonium (10 mM) and (□) K^+ (10 mM). (c) Effect of pinacidil on rings during contraction to (●) noradrenaline (100–300 nM), (○) noradrenaline (100–300 nM) in the presence of tetraethylammonium (10 mM) and (□) K^+ (20–30 mM). The relaxations are expressed as a percentage of the contraction and shown as means with s.e. mean indicated by vertical lines. Number of experiments per group: 5–7; *significantly different from the respective control ($P < 0.01$).

in which tone has been raised with K^+ (20–30 mM) were resistant to the relaxant effects of guanabenz. Similar findings have been found with other vasodilators such as pinacidil and BRL 34915 ((±)-6-cyano-3, 4-dihydro-2,2-dimethyl-*trans*-4-(2-oxo-1-pyrrolidyl-2H-benzo[b]pyran-3-ol)); both drugs depress noradrenaline-induced contractions but not those caused by K^+ (Hamilton et al 1986; Weston et al 1988; Videbaek et al 1988). It has been suggested that the mechanism through which pinacidil and BRL 34915 exert their relaxant effects is that of raising the membrane potentials close to their equilibrium potential for K^+ as a result of opening potassium channels. Another argument favouring the view that both guanabenz and pinacidil relax vascular smooth muscle cells through the opening of potassium channels is that tetraethylammonium, a drug known to block potassium channels (Southerton et al 1988), significantly reduced their relaxants effects.

Other explanations for the relaxant effects of guanabenz could be its interference with sodium channels or its action as an intracellular calcium antagonist. There is evidence that a number of vasoconstrictors, including noradrenaline (Owen 1986), activate the Na^+/H^+ exchange system in vascular smooth muscle cells, and amiloride, an inhibitor of Na^+/H^+ exchange, is a potent vasorelaxant drug (Reynolds et al 1988). Guanabenz, like amiloride, has also been suggested to be an inhibitor of the Na^+/H^+ exchange system (Frelin et al 1986). However, in contrast to amiloride, which relaxes rat aorta preparations preconstricted by potassium, guanabenz did not produce a similar effect. On the other hand, it is well documented that the vascular smooth muscle contractile effects of high concentrations of K^+ are completely dependent on calcium influx, whereas those exerted by noradrenaline can be due to both extracellular calcium and intracellular calcium mobilization (Bolton 1985). Therefore, it could be hypothesized that the tetraethylammonium-insensitive component of the relaxant effect induced by guanabenz would involve a mechanism involving inhibition of intracellular calcium mobilization. This has been recently described for KT-362 (5-(N-(2-(3,4-dimethoxyphenyl)ethyl)-β-alanyl)-2,3,4,5-tetra hydro-benzo-1,5-thiazepine bifumarate), a putative intracellular calcium antagonist, which only relaxes vascular smooth muscle preparations constricted with noradrenaline, but not by high K^+ (Eskinder et al 1989).

In conclusion, the results presented suggest that the relaxant effects of guanabenz are not related to the generation or release of an endothelial derived releasing factor or to an effect upon α- or β-adrenoceptors. It is possible, however, that the mechanisms responsible for the relaxation of smooth muscle by guanabenz would involve the opening of potassium channels or the antagonism of calcium mobilization from intracellular stores.

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Choline is an inhibitory modulator of cholinergic nerve function in guinea-pig colon

S. J. TROUT, D. E. BURLEIGH, *Department of Pharmacology, The London Hospital Medical College, Turner Street, London E1 2AD, UK*

Abstract—Using the novel smooth muscle-myenteric plexus (smmp) preparation from the guinea-pig colon, it has been possible to measure acetylcholine (ACh) overflow by a radiolabelling technique. The possibility that choline may be a modulator of cholinergic nerve activity in this preparation was investigated by observing its effects on electrically-evoked overflow of [^{3}H]acetylcholine. Choline (72, 144 μM), in concentrations that did not contract the smmp preparation, caused a depression of electrically-evoked [^{3}H]ACh overflow. This effect was unlikely to be due to actions on cholinesterase enzymes by end product inhibition. Choline also produced substantial non-muscarinic elevation of spontaneous [^{3}H]overflow. It is concluded that inhibitory modulation of enteric cholinergic nerve activity by presynaptic muscarinic receptors may not be exclusively mediated by the actions of acetylcholine.

A new smooth muscle-myenteric plexus (smmp) preparation of the guinea-pig colon has been devised and shown to be well suited to studying cholinergic nerve activity when using a radiolabelling technique to estimate acetylcholine (ACh) release (Trout 1986). For electrical stimuli of more than one pulse it was thought possible that choline, derived from metabolized ACh, may be involved in modulating transmitter release from the nerve terminal. Such an effect of choline has been demonstrated in the guinea-pig longitudinal muscle-myenteric plexus preparation where choline reduces ACh output by stimulating presynaptic muscarinic receptors (Kilbinger & Kruel 1981). Thus the present study was undertaken to determine whether choline functions as an inhibitory modulator of cholinergic nerve activity in the guinea-pig colon.

Materials and methods

Guinea-pig colonic smmp strips were prepared and set up as previously described (Trout 1986). Strips were incubated for 60 min at 37°C in Krebs fluid containing 4 μCi mL^{-1} of [^{3}H]choline chloride (spec. act. 15 Ci mmol^{-1}, Amersham) after which they were superfused at 2·2–2·4 mL min^{-1} with Krebs fluid containing hemicholinium-3 (34·8 μM). Electrical field stimulation (EFS; 10 Hz, 0·5 ms at 150 mA) was carried out during the first 30 min of the incubation period to maximize pre-loading of releasable [^{3}H]ACh stores (Szerb 1976). Incubation and superfusion (by displacement overflow) of individual smmp strips were carried out in the same 1·5 mL volume baths which were fitted with vertical platinum wire electrodes to allow EFS of the tissue from a constant current stimulator. After 90 min equilibration the preparations were stimulated (1 Hz, 0·5 ms, 240 pulses at 150 mA). Superfusion fluid was collected for 2 min periods and 0·5 mL aliquots removed from each sample for liquid scintillation counting. Sample collections, to measure 'basal release', were made before and after both EFS and choline addition, and again at the end of the experiment. Collections were made during and immediately after EFS stimulation and during exposure of the tissue to choline to measure 'stimulated' release. Efficiency of counting was determined automatically by the external standard channels ratio method. Radioactive content of superfusion samples was expressed as Bq g^{-1} and calculated as described previously (Burleigh 1988). The evoked release of radioactive material, collected during and after EFS and during exposure to choline, was calculated from the difference between the 'calculated' basal release and the release during stimulation (calculated basal release + evoked release). Calculated basal release was obtained by fitting a regression line through observed basal values.

The E_2/E_1 ratio was calculated by dividing the sum of evoked values of the second period of EFS (E_2) by the sum of evoked values of the first period (E_1).

The evoked release of tritiated material by EFS, after previous incubation of the tissue with [^{3}H]choline, accurately represents the [^{3}H]ACh release from guinea-pig smmp strips (Trout 1989). Following a modification of the methodology of Marchbanks &

Correspondence to: D. E. Burleigh, Department of Pharmacology, The London Hospital Medical College, Turner Street, London E1 2AD, UK.