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Vascular β-Adrenoceptor-mediated Relaxation and the Tone of the Tissue in Canine Arteries

R. BEGONHA, D. MOURA AND S. GUIMARÃES

Institute of Pharmacology and Therapeutics, Faculty of Medicine, 4200-Porto, Portugal

Abstract

The aim of the present investigation was to study the influence of the tone in the response to β-adrenoceptor activation of four different canine arteries: coronary, pulmonary, mesenteric and splenic.

Five different levels of tone were produced (of about 35, 50, 65, 80 and 95% of the maximum) by adding phenylephrine (0·6, 1·0, 2·0, 4·0, and 10 μM, respectively) to the bath.

In the coronary artery at spontaneous tone, low concentrations of noradrenaline or adrenaline (1–3 nM) caused either relaxation or contraction, while after induced tone, both noradrenaline and adrenaline caused concentration-dependent relaxations, noradrenaline being more potent (EC50 of 0·16 (0·13–0·20) and 0·38 (0·28–0·67) μM, respectively; n = 6; $P < 0·05$). Only in the coronary artery did isoprenaline relax the tissue irrespective of the previous level of tone. In all the other arteries, isoprenaline was able to cause concentration-dependent relaxations only if the previous tone was submaximal. At 80% of the maximum, isoprenaline caused relaxation in the mesenteric and pulmonary arteries, but in the splenic artery it caused relaxation only when the tone was of about 65% of the maximum or less.

While in the coronary artery atenolol and ICI-118,551 (erythro-DL 1(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol) were equipotent in antagonizing isoprenaline, noradrenaline and adrenaline, in the other vessels ICI-118,551 was from 58 (splenic artery) to 525 (mesenteric artery) times more potent than atenolol against the isoprenaline relaxant effect.

We conclude that: the tone of the vessel represents a critical factor deciding the sense of the response (coronary artery; low concentrations of noradrenaline or adrenaline), the level at which the relaxant effect is triggered (mesenteric = 80% vs splenic = 65%), and the magnitude of the relaxant effect (always). β_2-Adrenoceptors predominate in the mesenteric, pulmonary and splenic arteries, while β_1-adrenoceptors predominate in the coronary artery. It is unlikely that adrenaline is able to cause vasodilation in any of the vascular beds studied.

Besides varying from tissue to tissue (Bevan et al 1980), β-adrenoceptor distribution can vary with species (Fleisch et al 1970; Ikezono et al 1987), age (Fleisch 1981; Docherty 1990; Guimarães et al 1994) vessel size in one vascular bed (Bevan et al 1980; Guimarães et al 1993) and location along the length of a single vessel (Guimarães et al 1993).

The present investigation was undertaken to study β-adrenoceptor-mediated relaxation at different levels of tone in some arteries of the dog.

Materials and Methods

In the municipal dog pound, mongrel dogs, 8–16 kg in weight, of either sex, were anaesthetized with pentobarbitone sodium (30 mg kg^{-1} injected in the forelimb). Immediately after having been removed, the mesenteric, splenic, pulmonary and coronary arteries were placed in small vials containing aerated (95% O_2–5% CO_2) and frozen Krebs-Henseleit solution of the following composition (mM): NaCl 118·6, KCl 4·70, $CaCl_2$ 2·52, KH_2PO_4 1·18, $MgSO_4$ 1·23, $NaHCO_3$ 2·50, glucose 10, ascorbic acid 0·57, disodium EDTA 0·027 (Guimarães et al 1978). The animals were killed by an overdose of pentobarbitone sodium (100 mg kg^{-1}). The arteries were then transported to the laboratory where they were denuded from the endothelium by gentle rubbing of the internal surface with paper moistened in Krebs–Henseleit solution.

From each segment of artery four rings of about 6 mm length were cut and mounted between a stationary tissue holder and a transducer, and isometric contractions or relaxations were recorded as changes in N of tension on a Harvard Universal Oscillograph. The rings were allowed to stabilize for 1 h under basal tension before experimentation.

To study the role of β-adrenoceptor stimulation, concentration–response curves to isoprenaline were determined after the preparation had been contracted by phenylephrine. By using different concentrations of phenylephrine, five different levels of tone were produced: to about 95% (i.e. 90–100%) of maximum; to about 80% (i.e. 75–85%) of the maximum; to about 65% (i.e. 60–70%) of the maximum; to about 50% (i.e. 45–55%) of the maximum; and to about 35% (i.e. 30–40%) of the maximum. After the contraction had attained a steady state, concentration–response curves to isoprenaline (a β-adrenoceptor agonist) or forskolin (a direct activator of adenylate cyclase (Seamon & Daly 1981)) were obtained by increasing bath concentrations cumulatively by half-log increments. Two concentration–response curves were determined per ring. To study the antagonism exerted by atenolol (a selective β_1-adrenoceptor antagonist (Barrett et al 1973))

Correspondence: S. Guimarães, Institute of Pharmacology, Faculty of Medicine, 4200-Porto, Portugal.

and by the compound ICI-118,551 (a selective β_2-adrenoceptor antagonist (Bilski et al 1980)) on the effect of isoprenaline, the drug was added to the bathing fluid 25 min before the second concentration-response curve. In all experiments cocaine (12 μM, to inhibit neuronal uptake, Trendelenburg (1966)), hydrocortisone (41 μM) and U-0521 (40 μM, to reduce uptake$_2$ and *O*-methylation, Guimarães et al (1978)) were present in the bathing solution.

The results are presented as geometric means with confidence limits or as arithmetic means with their standard errors. Differences between means were compared by Student's *t*-test for unpaired or paired data.

Drugs used were: (±)-atenolol hydrochloride (ICI, Macclesfield, UK); cocaine hydrochloride (Uquipa, Lisboa, Portugal); forskolin (Sigma, St Louis, MO, USA); hydrocortisone 21-hemisuccinate sodium (Sigma); (±)-ICI-118,551 hydrochloride (erythro-DL 1(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol) (ICI); (−)-isoprenaline bitartrate (Sigma); (−)-phenylephrine hydrochloride (Boehringer Sohn, Mannheim, Germany); and U-0521 (3,4-dihydroxy-2-methylpropiophenone (Upjohn, Kalamazoo, USA)).

Results

Coronary arteries

In the absence of induced tone, isoprenaline caused concentration-dependent relaxations but its maximal effect was relatively small: 0·03 ± 0·01 N g^{-1} (n = 6). In the lowest effective concentration (1–3 nM) both adrenaline and noradrenaline caused either relaxation or contraction. However, as the concentration increased (3–10 nM), a triphasic response developed; contraction followed by relaxation followed by contraction (Fig. 1).

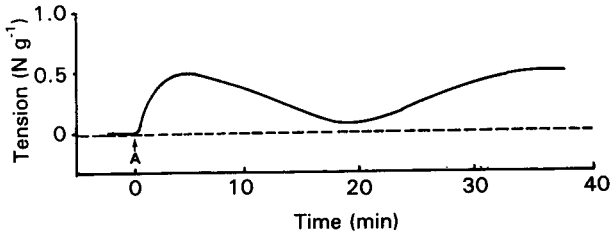

FIG. 1. Coronary artery. A typical response to 5 nM adrenaline at spontaneous tone is shown.

After the tone had been elevated by phenylephrine, isoprenaline caused concentration-dependent relaxations even when the level of the previous tone was maximal and its maximal effect was larger than the previously induced tone, such that the baseline after the maximal effect of isoprenaline was below the initial baseline (Table 1). In the presence of tone, both adrenaline and noradrenaline caused concentration-dependent relaxations with a maximal effect which sometimes did not fully antagonize the previous tone. In these cases, when the relaxant effect approached about 15% of the maximum a slowly developing contraction appeared.

As β-adrenoceptor agonist, noradrenaline was slightly more potent than adrenaline (EC50 of 0·16 (0·13–0·20) μM and 0·38 (0·22–0·67) μM for noradrenaline and adrenaline, respectively; n = 6; $P < 0·05$).

Mesenteric, splenic and pulmonary arteries

In the absence of tone none of the sympathomimetic amines was able to cause relaxation of the mesenteric, splenic or pulmonary arterial tissue.

As shown in Table 1, when the tone was elevated by

Table 1. Maximal relaxant effect of isoprenaline after contractions of different magnitude caused by phenylephrine.

Artery used	Magnitude of the contraction to phenylephrine (% of the maximal effect)	Tension developed (N)	Maximal relaxation to isoprenaline	n
Coronary	95 (90–100)	2·8 ± 0·3	3·1 ± 0·4	6
Mesenteric	95 (90–100)	14·3 ± 1·5	No relaxation	4
Splenic	95 (90–100)	11·2 ± 3·1	No relaxation	4
Pulmonary	95 (90–100)	3·2 ± 0·6	No relaxation	4
Coronary	80 (75–80)	2·2 ± 0·4	2·4 ± 0·4	5
Mesenteric	80 (75–80)	11·4 ± 2·0	3·4 ± 0·3	4
Splenic	80 (75–80)	7·2 ± 1·5	No relaxation	4
Pulmonary	80 (75–80)	2·6 ± 0·4	0·4 ± 0·1	6
Coronary	65 (60–70)	1·9 ± 0·3	2·0 ± 0·2	6
Mesenteric	65 (60–70)	8·0 ± 2·3	5·6 ± 0·8	5
Splenic	65 (60–70)	5·0 ± 0·7	0·8 ± 0·2	5
Pulmonary	65 (60–70)	1·8 ± 0·3	0·3 ± 0·1	5
Coronary	50 (45–55)	1·5 ± 0·3	1·7 ± 0·2	5
Mesenteric	50 (45–55)	5·8 ± 1·1	4·8 ± 0·9	4
Splenic	50 (45–55)	3·8 ± 0·5	2·9 ± 0·4	5
Pulmonary	50 (45–55)	1·6 ± 0·3	0·5 ± 0·1	5
Coronary	35 (30–40)	1·0 ± 0·2	1·1 ± 0·2	5
Mesenteric	35 (30–40)	3·1 ± 0·7	2·7 ± 0·2	6
Splenic	35 (30–40)	2·2 ± 0·4	2·1 ± 0·4	5
Pulmonary	35 (30–40)	1·4 ± 0·3	0·7 ± 0·2	5

The mean weight of the rings was 9 mg. The concentration of phenylephrine required to obtain 35, 50, 65, 80 and 95% of the maximal tone were 0·6, 1·0, 2·0, 4·0 and 10·0 μM, respectively.

Table 2. Antagonism (pA_2 values ± s.e.m.) exerted by the selected β_1-adrenoceptor antagonist atenolol and by the selective β_2-adrenoceptor antagonist the compound ICI-118,551 against the relaxant effect of isoprenaline in four different canine arteries.

Artery	Atenolol	n	ICI-118,551	n
Mesenteric	6.59 ± 0.09^a	5	9.31 ± 0.13^b	5
Splenic	7.13 ± 0.22^c	4	8.90 ± 0.27^d	5
Pulmonary	6.89 ± 0.18^e	7	9.50 ± 0.24^f	6
Coronary	8.03 ± 0.18^g	4	7.53 ± 0.33^h	4

Significant differences between: [a] and [b]; [a] and [c]; [c] and [d]; [e] and [f]; [e] and [g]; [g] and [a]; [f] and [h] were found.

phenylephrine, isoprenaline was able to cause concentration-dependent relaxations in all the arteries whenever its level was sub-maximal. At 80% of maximal tone, isoprenaline caused concentration-dependent relaxations in the mesenteric and in the pulmonary arteries, but it relaxed the splenic artery only when the tone of this vessel was reduced to about 65% of the maximum.

In spite of the marked difference of force developed by the contraction of the different arteries (and of the corresponding relaxations), the EC50 for isoprenaline was not significantly different in the different vessels.

In contrast to isoprenaline, forskolin (0·05–5 μM) caused concentration-dependent relaxations and its maximal effect always completely antagonized the previously induced contraction.

Influence of atenolol and ICI-118,551

As shown in Table 2, in the mesenteric, splenic and pulmonary arteries, the selective β_2-adrenoceptor antagonist ICI-118,551 was much more potent than atenolol at antagonizing the isoprenaline effect. The relative pA_2 values for either antagonist was different from vessel to vessel, showing that the relative contribution of β_1- and β_2-adrenoceptors to the response is different in the different arteries.

In the coronary artery, atenolol and ICI-118,551 were equipotent.

Discussion

Adrenaline is a potent agonist at both α- and β-adrenoceptors. In-vivo, the final balance between α- and β-adrenoceptor-mediated action of adrenaline at the cardiovascular level was shown by the classical experiments of Dale (1906). Under control conditions, α-adrenoceptor-mediated responses predominate such that a pressor response is obtained when adrenaline is intravenously injected. However, the previous administration of an α-adrenoceptor antagonist converts this response into a depressor response. Similarly, in isolated vessels in-vitro, it was shown that α-adrenoceptor-mediated effect (contraction) predominates in the absence and is converted into a β-adrenoceptor-mediated effect (relaxation) in the presence of an α-adrenoceptor antagonist (Guimarães 1975; Guimarães & Paiva 1981). In all arteries used in the present study there are both α- and β-adrenoceptors. However, only in the coronary artery did β-adrenoceptor stimulation by isoprenaline generate a relaxation strong enough to completely counterbalance the maximal contraction previously caused by phenylephrine. In the mesenteric and pulmonary arteries, β-adrenoceptor stimulation only partially antagonized the previous contraction if it had not reached more than about 80% of the maximum, and in the splenic artery the relaxant effect only appeared when the tone was about 65% of the maximum or less. Furthermore, in the coronary artery, while adrenaline and noradrenaline were able to cause concentration-dependent relaxations which almost fully antagonized the previous contraction irrespective of the previous level of tone, in the other arteries both amines caused an additional contraction to the previous one elicited by phenylephrine. These two facts clearly show that β-adrenoceptor population plays a much more important role in the coronary than in the other arteries. Another difference between the coronary and the other arteries was that while in the mesenteric, splenic and pulmonary arteries β_2-adrenoceptors predominate, in the coronary artery β_1-adrenoceptors are largely predominant or even exclusive. In fact, while in the systemic arteries the selective β_2-adrenoceptor antagonist ICI-118,551 was much more potent than atenolol at antagonizing the responses to isoprenaline, in the coronary artery, ICI-118,551 and atenolol were about equally potent. Additionally, the fact that noradrenaline was more potent than adrenaline as a β-adrenoceptor stimulant confirms that β-adrenoceptors of the coronary artery belong to the β_1-subtype (Baron et al 1972; De la Lande et al 1974).

It is interesting that some years ago De Mey & Vanhoutte (1982) reported that canine arteries (splenic, pulmonary, femoral, and saphenous) do not relax when exposed to isoprenaline. One difference between their and our experimental conditions was that the blood vessel rings were made to contract by noradrenaline in their experiments and by phenylephrine in ours. Since phenylephrine is practically devoid of β-effects, it may be that β-adrenoceptors are left totally free to be activated when phenylephrine is used to induce tone and partially or totally occupied when noradrenaline is the agent-inducing tone (Guimarães 1975).

The role of the tone in the apparent effect of β-adrenoceptor stimulation in the vascular tissue is clearly shown by the present study and can be exemplified by the results obtained in the coronary arteries. In absence of tone, 3–10 nM adrenaline caused a triphasic response: an initial contraction, followed by a relaxation of about the same magnitude as the previous contraction, which was itself followed by a second contraction. This balance between contraction and relaxation shows very clearly that the tone represents the critical factor deciding the sense of the response.

For many years it has been accepted, at least in man, that splanchnic and skeletal muscle vascular beds dilate to adrenaline because β-adrenoceptors predominate in their vessels (Hoffman & Lefkowitz 1990). In the present study no important β-adrenoceptor-mediated responses were obtained in the systemic arteries of dog, even in those belonging to the splanchnic circulation. It is known that small and large vessels do not necessarily have identical adrenoceptor distribution (Guimarães 1986). For example, large coronary vessels exhibit both α- and β-adrenoceptor-mediated responses as the present results show, while the small vessels of the coronary circulation exhibit only

β-adrenoceptor-mediated responses (Bevan et al 1975). It may be that in the dog, β-adrenoceptors are associated only with small arterioles and precapillary sphincters not available for studies in-vitro. However, in experiments in which the hind-limb of the dog was perfused, adrenaline, in spite of reaching smaller arteries and precapillary sphincters, caused concentration-dependent increases of the perfusion pressure, showing that α-adrenoceptor-mediated vasopressor effects predominate in this vascular bed (Teixeira 1977). Thus, it appears that in the dog, in contrast to what happens in man, even in skeletal muscle arteries, α-adrenoceptor-mediated responses predominate.

In summary, the present results indicate that in the canine arteries studied, with the exception of the coronary artery, the role played by β-adrenoceptor stimulation is relatively poor; the tone of the vessels represents a critical factor deciding the sense of the response (for low concentrations of noradrenaline or adrenaline in the coronary artery), the level at which the relaxant effect starts (80% in the mesenteric vs 65% in the splenic artery), and the magnitude of the relaxant response (always); and β_2-adrenoceptors predominate in the mesenteric, pulmonary and splenic arteries, while β_1-adrenoceptors predominate in the coronary artery.

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