β_1-Adrenoceptor-Mediated Relaxation by Norepinephrine in Dog Hepatic Arteries

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ABSTRACT—Dog hepatic arterial strips treated with prazosin responded to norepinephrine with concentration-related, endothelium-independent relaxations, the maximal response being 81.7% of the papaverine-induced maximal relaxation that was markedly greater than that in renal arteries. The norepinephrine-induced relaxation in hepatic arteries was significantly attenuated by metoprolol but not influenced by butoxamine. Relaxant responses to norepinephrine of dog hepatic arteries appear to be mediated by the β_1-adrenoceptor subtype, like those of coronary arteries. Evidence for functioning of the β_1-subtype in hepatic arteries would contribute to the analysis of neural and hormonal regulation of blood flow in the liver.

Keywords: Hepatic artery, Norepinephrine, β_1-Adrenoceptor

The hepatic vasculature seems to be innervated exclusively by sympathetic nerves (1). The major role of the nerve is to constrict hepatic arteries and arterioles by a mediation of α-adrenoceptors that are stimulated by neurogenic norepinephrine. However, there is some evidence for the presence of β-adrenoceptors in hepatic vasculatures. The increase of hepatic blood flow in anesthetized cats (2－4) and isolated canine perfused livers (1) following administration of isoproterenol is prevented by β-receptor antagonists. Vasodilator responses to norepinephrine of hepatic vasculature in anesthetized dogs are not blocked by atenolol, a β_1-receptor antagonist, but stimulated by salbutamol, a β_2-agonist, (5). However, there have been no detailed analyses of the response mediated by β-adrenoceptors in isolated hepatic arteries. Therefore, the present study was undertaken to compare the response to norepinephrine of dog hepatic arteries under α-adrenoceptor blockade and to determine the β-adrenoceptor subtypes involved in the norepinephrine-induced relaxation. Responses of the isolated renal artery were also examined for comparison.

The studies review board at our university approved the use of animal blood vessels in this study. Mongrel dogs of either sex, weighing 7 to 16 kg, were anesthetized with i.v. injections of sodium pentobarbital (30 mg/kg) and sacrificed by bleeding from the carotid arteries. The liver and kidney were rapidly removed, and hepatic (1.0－1.4 mm outside diameter) and renal (1.0－1.2 mm) arteries were isolated. The arteries with endothelium were cut helically into strips of approximately 20 mm in length. The specimens were fixed vertically between hooks in a muscle bath containing the modified Ringer-Locke solution kept at 37±0.3℃ and aerated with a mixture of 95% O_2 and 5% CO_2. Resting tensions of the hepatic and renal arterial strips were adjusted to 1.5 g. The isometric tension was recorded via a force-displacement transducer. Responses to norepinephrine were obtained in the arterial strips partially contracted with prostaglandin (PG) $F_{2\alpha}$. Concentration-response curves for norepinephrine were obtained from the strips treated with 10^{-5} M prazosin by adding the amine directly to the bathing media in cumulative concentrations. At the end, papaverine (10^{-4} M) was applied to attain the maximal relaxation, and the norepinephrine-induced relaxation relative to that induced by papaverine was presented. In some experiments, the endothelium of the arterial strips were removed by gently rubbing the intimal surface with a cotton pellet, and the strips were used for comparison. Successful removal of the endothelium was confirmed by abolition of the relaxation produced by the calcium ionophore A23187. After the responses to norepinephrine in the first and second trials were confirmed to be identical, the arterial strips were treated for 30 min with metoprolol or butoxamine. The dissociation constant (K_B) of

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metoprolol was determined according to the following equation: $K_B = [B] / (\text{dose ratio} - 1)$, where B is the concentration of metoprolol, and the dose ratio is the ratio of median effective concentrations (EC_{50}) of norepinephrine in the presence and absence of the antagonist. The results shown in the text, figure and table are expressed as mean values±S.E.M. The EC_{50} values are expressed as geometric means and the range of the values. Statistical analyses were made by the unpaired Student's *t*-test or Tukey's method after one-way analysis of variance.

In the hepatic arterial endothelium-intact strips treated with 10^{-5} M prazosin and partially contracted with $PGF_{2\alpha}$, the addition of norepinephrine (10^{-9} to 5×10^{-5} M) produced a concentration-related relaxation, which was not affected by endothelium denudation. The EC_{50} values of the response of the strips with and without endothelium were $8.89\ (7.42-10.6)\times10^{-8}$ M (n=14) (Table 1) and $7.35\ (4.94-10.9)\times10^{-8}$ M (n=4), respectively.

Table 1. Mean values of the maximal relaxation (MR) induced by and the apparent median effective concentration (EC_{50}) of norepinephrine in dog hepatic, renal, coronary and mesenteric arteries treated with α-receptor antagonists

Artery	N	MR (%)[a]	EC_{50} ($\times10^{-7}$ M)	Reference
Hepatic	14	81.7±2.8	0.89 (0.74-1.06)	Present study
Renal	4	23.6±3.6[b,c]	0.70 (0.37-1.33)	Present study
Coronary	7	85.1±1.9	0.57 (0.49-0.66)	(6)
Mesenteric	7	16.1±5.5[b,c]	7.39 (5.33-10.2)	(6)

N, number of preparations used. [a]Relaxation relative to that induced by 10^{-4} M papaverine. Significantly different from the relaxant response of hepatic arteries: [b]$P<0.01$; different from those of coronary arteries: [c]$P<0.01$ (Tukey's method).

The maximal relaxations were 81.7±2.8% (n=14) (Table 1) and 82.0±6.4% (n=4), respectively.

The concentration-response curve for norepinephrine

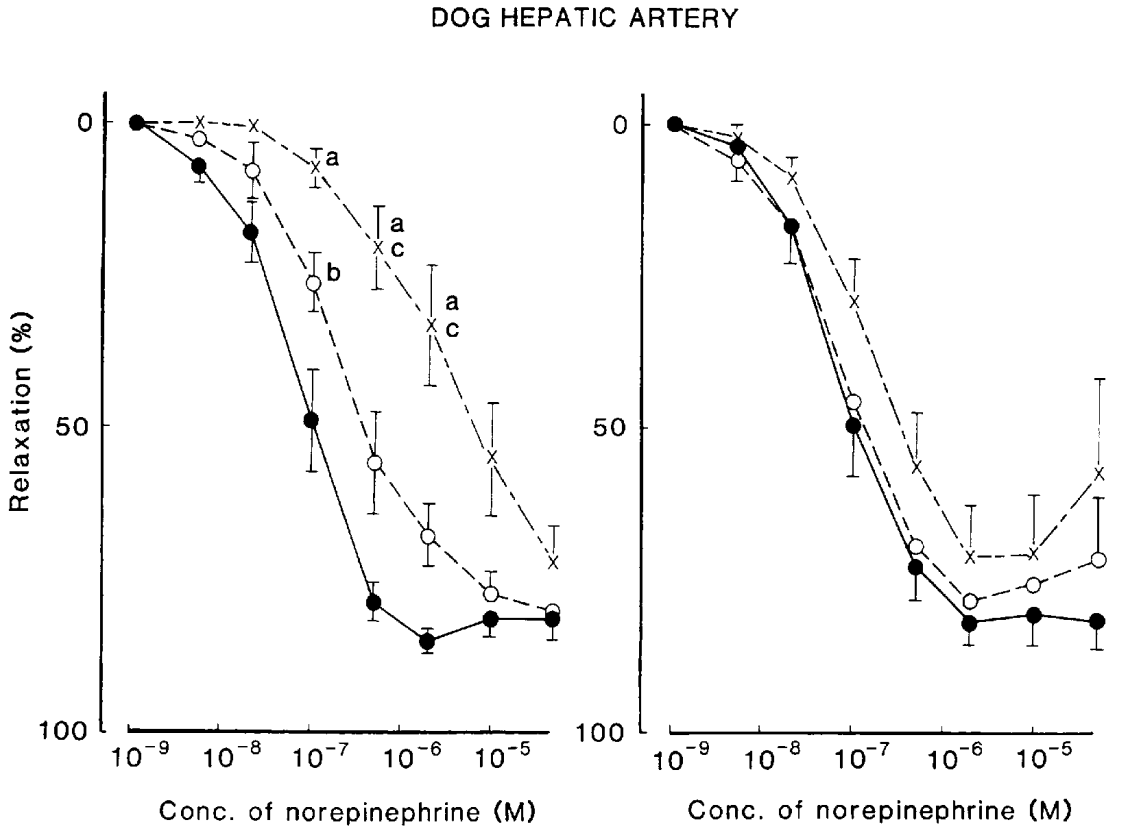

Fig. 1. Modification by metoprolol (left panel) and butoxamine (right) of the relaxant response to norepinephrine of hepatic arteries treated with 10^{-5} M prazosin. Preparations were precontracted with $PGF_{2\alpha}$. Relaxations induced by 10^{-4} M papaverine were taken as 100%; mean absolute values in the control arteries (●) and those treated with 5×10^{-8} (○) and 2×10^{-7} M (×) metoprolol were 237±31 (n=7), 194±22 (n=7) and 241±29 mg (n=7), respectively; and those in the control arteries (●) and those treated with 10^{-6} (○) and 10^{-5} M (×) butoxamine were 227±24 (n=7), 277±40 (n=7) and 259±27 mg (n=7), respectively. Significantly different from the control: [a]$P<0.01$, [b]$P<0.05$; significantly different from those treated with 5×10^{-8} M: [c]$P<0.01$ (Tukey's method).

of the hepatic arterial strips was shifted to the right by treatment with metoprolol (5×10^{-8} and 2×10^{-7} M) in a concentration-dependent manner (Fig. 1, left), but was not significantly influenced by butoxamine (10^{-6} and 10^{-5} M) (Fig. 1, right). The K_B value of metoprolol at 2×10^{-7} M was $[7.12\pm1.81]\times10^{-9}$ M.

In the renal arterial strips treated with 10^{-5} M prazosin and partially contracted with $PGF_{2\alpha}$, norepinephrine (10^{-9} to 5×10^{-5} M) elicited only slight relaxation (n=4). The EC_{50} value of the response was 6.98 (3.65–13.3) $\times10^{-8}$ M (n=4). The maximal relaxation averaged 23.6±3.6% (n=4), the value being significantly smaller than that seen in the hepatic arteries ($P<0.001$) (Table 1).

The present study revealed that, under α_1-adrenoceptor blockade, norepinephrine produced marked endothelium-independent relaxations (maximum: about 82% of the papaverine-induced relaxation) in the dog hepatic arterial strips but only a slight relaxation in the renal artery (about 24%). Similar contrasting responsiveness to norepinephrine was also observed in dog coronary and mesenteric arteries (6). It is well recognized that isolated coronary arteries respond to norepinephrine with profound relaxations via β_1-adrenoceptors. Arteries of other vascular beds do not or only slightly relax in response to norepinephrine, but respond well to isoproterenol. The reason for the discrepancy between these arteries is explained by the relatively selective action of norepinephrine on the β_1-receptor subtype (7) and by predominant distribution of the β_2-subtype in vasculatures (8) other than the coronary artery. However, it was a surprise to see that the dog hepatic arterial response to norepinephrine was almost identical to that of coronary arteries (Table 1).

The norepinephrine-induced relaxation in hepatic arterial strips was attenuated by metoprolol, a selective β_1-antagonist (9), whereas butoxamine, a β_2-receptor antagonist (10), was without significant effect. These findings, suggest that the norepinephrine-induced relaxation in dog hepatic arteries is mediated by the β_1-, but not β_2-adrenoceptor subtype. This is in contrast to the results obtained in anesthetized dogs, in which a decreased hepatic vasculature resistance by intraarterial isoproterenol is not blocked by a β_1-blocker (5). The discrepant results may be due to different experimental methods (isolated vs in vivo), different drugs (norepinephrine vs isoproterenol) and different portions of vasculature (proximal artery vs resistance vessel). The functioning of β_1-adrenoceptor subtype of hepatic arteries, involved in vasodilatation, would be quite important to analyze mechanisms of the regulation by autonomic nerves of hepatic blood flow, because the tone of adrenergically-innervated arteries is controlled by a balance of α_1-mediated constriction and β_1-mediated dilatation that are induced by norepinephrine.

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