

## PROFILE OF $\beta$ -ADRENOCEPTORS IN FEMORAL, SUPERIOR MESENTERIC AND RENAL VASCULAR BEDS OF DOGS

N. TAIRA & Y. YABUUCHI

Department of Pharmacology, Tohoku University School of Medicine, Sendai 980, Japan

S. YAMASHITA

Biological Research Laboratories, Otsuka Pharmaceutical Co.,  
Kawauchi, Tokushima 771-01, Japan

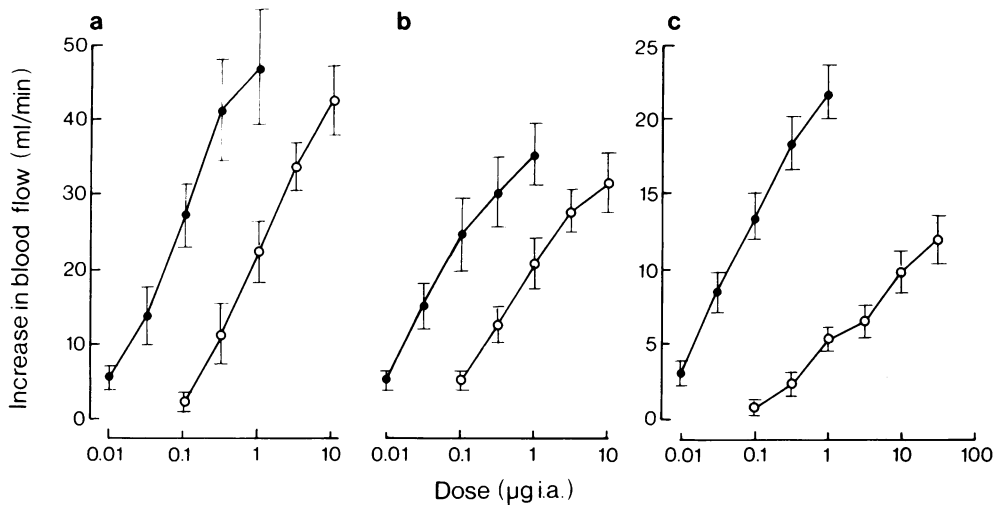
- 1 The homogeneity of  $\beta$ -adrenoceptors in femoral, superior mesenteric and renal vascular beds was investigated by the use of the regional perfusion technique in dogs.
- 2 Isoprenaline and salbutamol produced dose-related increases in femoral and superior mesenteric blood flow. The dose-response curves for the two agonists were parallel, but salbutamol was approximately 1/15 as potent as isoprenaline on a weight basis.
- 3 Isoprenaline and salbutamol increased renal blood flow in a dose-related manner. However, salbutamol was approximately 1/240 as potent as isoprenaline on a weight basis, and the slope of the dose-response curve for salbutamol was less steep than that for isoprenaline.
- 4 The dose-response curves to isoprenaline for increase in femoral and superior mesenteric blood flow were shifted to the right by intravenous pindolol but not by intravenous or intra-arterial practolol.
- 5 The dose-response curves to isoprenaline for increase in renal blood flow were shifted to the right more markedly by intravenous pindolol than by intravenous or intra-arterial practolol.
- 6 The results indicate that  $\beta$ -adrenoceptors of the renal vascular bed consist of  $\beta_1$ -type and  $\beta_2$ -type whereas the femoral and superior mesenteric vascular beds contain only  $\beta_2$ -adrenoceptors.

### Introduction

Lands and his co-workers (Lands, Arnold, McAuliff, Luduena & Brown, 1967a; Lands, Luduena & Buzzo, 1967b) proposed that  $\beta$ -adrenoceptors could be divided into a  $\beta_1$ -group in cardiac muscle and a  $\beta_2$ -group in bronchial and vascular smooth muscle. Recent work suggests that not only do  $\beta$ -adrenoceptors in bronchial and vascular smooth muscle differ from those in cardiac muscle but that  $\beta$ -adrenoceptors in the former two sites are also not homogeneous (Bristow, Sherrod & Green, 1970; Wasserman & Levy, 1974; Wardell, Colella, Shetzline & Fowler, 1974). Little is known about the homogeneity of  $\beta$ -adrenoceptors in the vasculature. The present experiments were designed to test this point. For this purpose the femoral, superior mesenteric and renal vascular beds were perfused by the use of the regional perfusion technique (Hashimoto & Kumakura, 1965) in dogs anaesthetized with pentobarbitone.

### Methods

Adult mongrel dogs of either sex, weighing 15-18 kg, were anaesthetized with pentobarbitone sodium initially at a dose of 30 mg/kg (i.v.) and received heparin sodium at a dose of 500 units/kg (i.v.). Subsequently pentobarbitone sodium was infused intravenously at a rate of 4 mg kg<sup>-1</sup> h<sup>-1</sup> and heparin sodium at a rate of 100 units kg<sup>-1</sup> h<sup>-1</sup> by the use of an infusion pump (Harvard Apparatus, Model 600). The number of dogs used was as follows: 26 for experiments on renal circulation, 15 for those on superior mesenteric circulation and 15 for those on femoral circulation. The surgical procedures were essentially the same as those described by Hashimoto & Kumakura (1965). The left renal, superior mesenteric or right femoral artery was cannulated and blood from the right femoral artery was conducted to the cannulated artery by means of a peristaltic pump (Harvard Apparatus, Model 1210). Constant pressure perfusion was achieved by the use of Starling



**Figure 1** Dose-response curves to intra-arterial isoprenaline (●) and salbutamol (○) for peak increase in (a) femoral, (b) superior mesenteric and (c) renal blood flow in dogs. Each symbol represents the mean and vertical bars show s.e. mean. Number of experiments is 11 for isoprenaline in renal circulation and 5 in the remainder.

pneumatic resistance through which a portion of blood was shunted to the left jugular vein. Perfusion pressure was maintained at 120 mmHg for renal circulation and at 100 mmHg for superior mesenteric circulation and for femoral circulation. Blood flow in each case was measured by an electromagnetic flowmeter (Nihon Koden, MF-26). The systemic blood pressure was measured from the left carotid artery by a pressure transducer (San-ei Instrument, MPU-0.5) and the heart rate by a cardi tachometer (San-ei Instrument, Type 2130) triggered by blood pressure pulses or R waves of the ECG. All the recordings were made on an ink-writing rectigraph (San-ei Instrument, Rectihoriz Type 8S).

The drugs used in this study were (–)-isoprenaline hydrochloride (Nikken Kagaku), (±)-salbutamol sulphate (Leiras), (±)-noradrenaline hydrochloride (Sankyo), (±)-pindolol (Sandoz), (±)-practolol (ICI) and phenoxybenzamine hydrochloride (Nakarai Kagaku). All drugs except for pindolol and practolol were dissolved in 0.9% w/v NaCl solution (saline). Pindolol was dissolved in equimolar maleic acid solution and practolol in equimolar HCl. All drug solutions were diluted with saline to the desired concentrations. Doses of all drugs except for pindolol and practolol refer to their salts, and those of the latter two drugs to their bases. Agonist solutions in a volume of 10–30 µl were injected into the rubber tubing connected to the arterial cannula within 3 s by the use of microsyringes. Antagonist solutions were infused at a rate of 0.2 ml/min into the rubber tubing connected to the arterial cannula by the aid of an infusion pump (Harvard Apparatus, Model 600) or injected into the forearm vein (0.4–1.0 ml in 10 s).

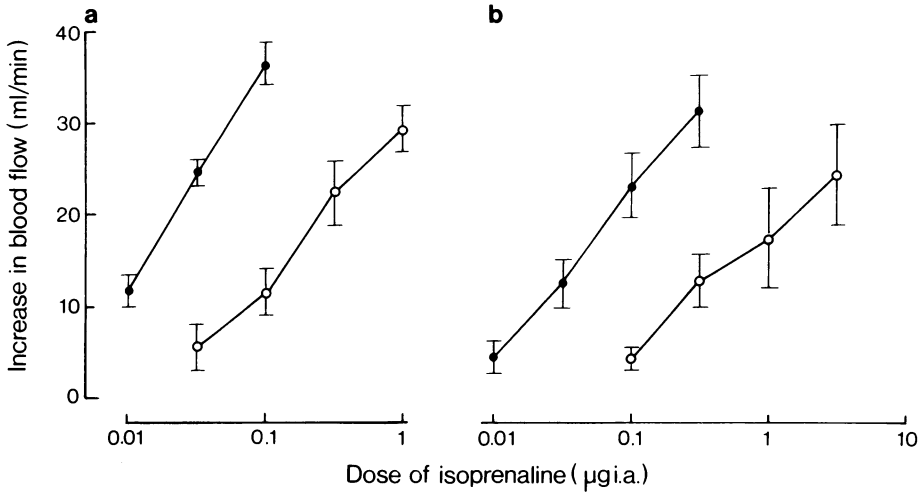
Values in the text are arithmetic means  $\pm$  s.e. The difference between mean values was analysed by Student's *t* test and judged to be significant when *P* values  $< 0.05$ .

## Results

### *Effects of isoprenaline and salbutamol on femoral, superior mesenteric and renal blood flow*

Femoral and superior mesenteric blood flow at a perfusion pressure of 100 mmHg was  $46 \pm 7$  ml/min ( $n = 15$ ) and  $108 \pm 7$  ml/min ( $n = 15$ ), respectively. Intra-arterial injections of isoprenaline (0.01–1 µg) and of salbutamol (0.1–10 µg) increased blood flow through both arteries in a dose-related manner. Dose-response curves to isoprenaline and to salbutamol for peak increases in blood flow were parallel in both circulations (Figure 1a,b). When compared on the basis of doses (ED, 20 ml/min) increasing blood flow by 20 ml/min, the potency of salbutamol was almost 1/15 that of isoprenaline on a weight basis in the two vascular beds.

In all experiments on renal circulation the animals were initially given phenoxybenzamine (2–5 mg/kg i.v.). Under these conditions renal blood flow at a perfusion pressure of 120 mmHg was  $106 \pm 4$  ml/min ( $n = 26$ ) and decreases in renal blood flow in response to noradrenaline (0.1–0.3 µg i.a.) were greatly diminished. Intra-arterial injections of isoprenaline (0.01–1 µg) and of salbutamol (0.3–30 µg) produced a dose-related increase in the



**Figure 2** Dose-response curves to isoprenaline for increase in (a) femoral blood flow and (b) superior mesenteric blood flow before (●) and after (○) a single injection of pindolol (3  $\mu\text{g}/\text{kg}$  i.v.). Vertical bars show s.e. mean ( $n=5$ ).

renal blood flow. Figure 1c shows dose-response curves to the two agonists for peak increase in blood flow. As seen in this figure, the slope of the dose-response curve for salbutamol was less steep than that for isoprenaline. When compared on the basis of doses (ED, 10 ml/min) increasing the renal blood flow by 10 ml/min, the potency of salbutamol was approximately 1/240 that of isoprenaline on a weight basis. These doses, apart from the highest doses used of isoprenaline and salbutamol injected intra-arterially, had almost no systemic effect.

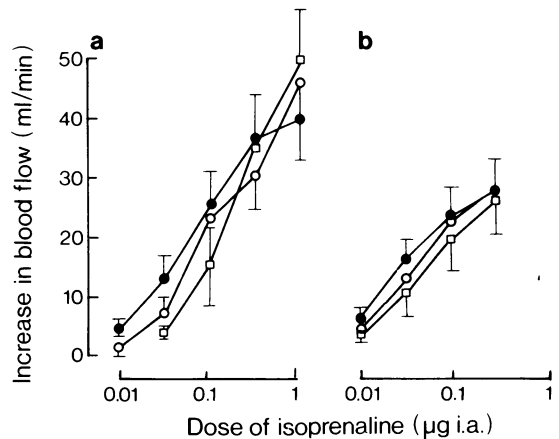
*Differential modification by pindolol and practolol of the effect of isoprenaline on femoral and superior mesenteric blood flow*

The dose-response curves to isoprenaline for increases in femoral and superior mesenteric blood flow were shifted almost in a parallel fashion to the right by intravenous injections of pindolol (3  $\mu\text{g}/\text{kg}$ ) (Figure 2a,b). In contrast, the dose-response curves to isoprenaline for increase in femoral blood flow were not altered significantly by intravenous injections of practolol (0.3 and 3 mg/kg) (Figure 3a). The dose-response curves to isoprenaline for increase in superior mesenteric blood flow were also not modified by intra-arterial infusions of practolol (100 and 300  $\mu\text{g}/\text{min}$ ) (Figure 3b).

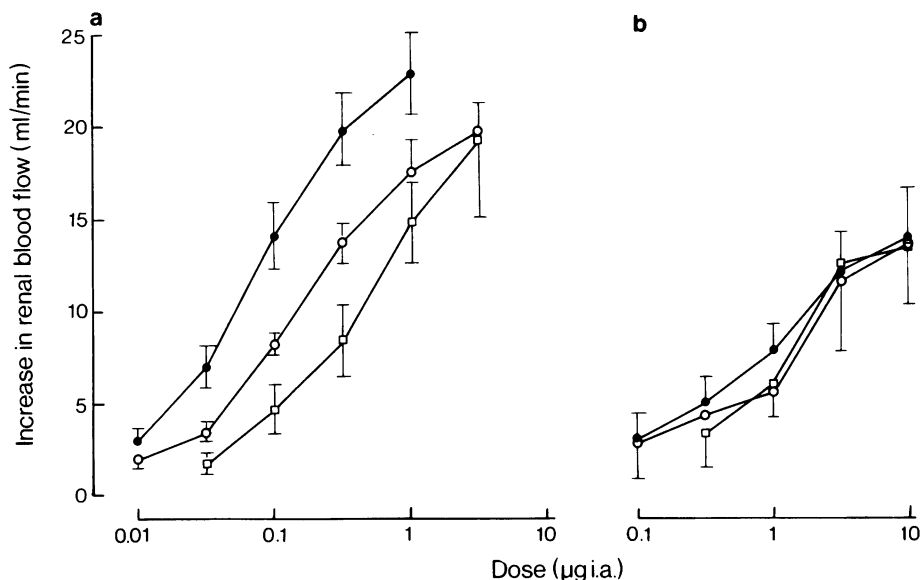
*Differential modification by practolol and pindolol of the effects of isoprenaline and salbutamol on renal blood flow*

Intravenous injections of practolol (0.3 and 3 mg/kg), which antagonized the positive chronotropic effect of

0.1  $\mu\text{g}/\text{kg}$  of intravenous isoprenaline by 60 to 100% but did not significantly alter the vasodepressor effect, shifted the dose-response curve to isoprenaline (0.01–3  $\mu\text{g}$  i.a.) for increase in renal blood flow in an almost parallel fashion to the right (Figure 4a) but did not alter that to salbutamol (0.1–10  $\mu\text{g}$  i.a.) (Figure 4b). Log ratios of ED, 10 ml/min for isoprenaline after 0.3 and 3 mg/kg of intravenous practolol to ED, 10 ml/min before practolol were 0.3 and 0.7



**Figure 3** (a) Dose-response curves to isoprenaline for increase in femoral blood flow before (●) and after single injections of practolol: (○) 0.3 mg/kg i.v.; (□) 3 mg/kg i.v. (b) Similar curves for increase in superior mesenteric blood flow before (●) and during infusion of practolol into the superior mesenteric artery: (○) 100  $\mu\text{g}/\text{min}$ ; (□) 300  $\mu\text{g}/\text{min}$ . Vertical bars show s.e. mean ( $n=5$ ).



**Figure 4** Dose-response curves to (a) isoprenaline and (b) salbutamol for increase in renal blood flow before (●) and after practolol: (○) 0.3 mg/kg i.v.; (□) 3 mg/kg i.v. Vertical bars show s.e. mean ( $n=5$ ).

respectively (Table 1), indicating that the doses of practolol produced shifts of 0.3 and 0.7 log units of the dose-response curves for isoprenaline to the right. Essentially similar antagonism of renal blood flow responses to isoprenaline (0.03–0.3  $\mu\text{g}$  i.a.) was obtained with intra-arterial infusions of practolol (10–300  $\mu\text{g}/\text{min}$ ) (Table 1). Figure 5 illustrates typical experiments. The blocking effect of practolol was

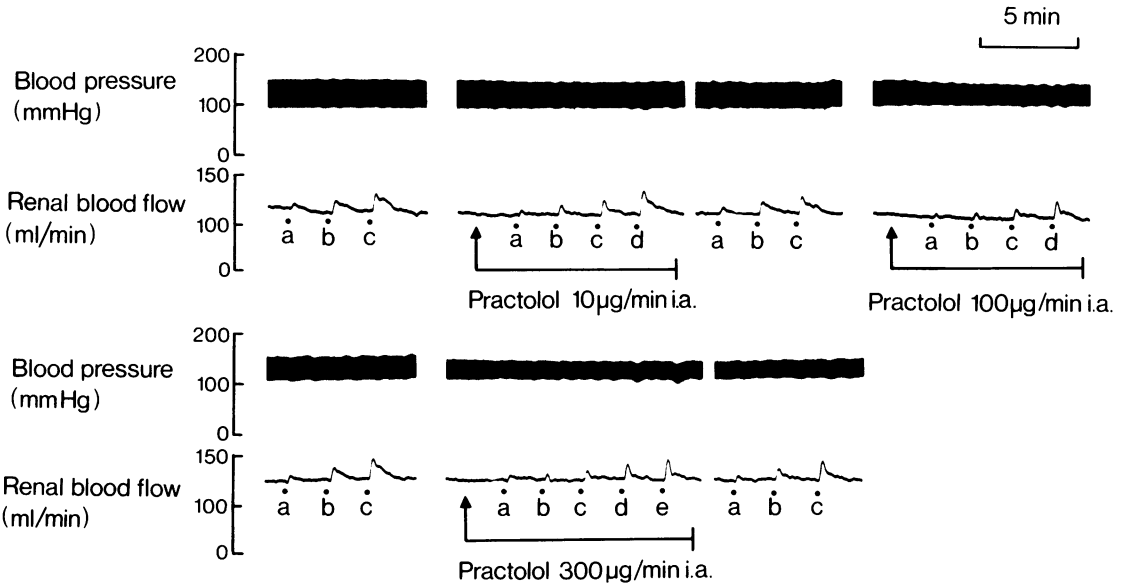
reversible; the blood flow response to isoprenaline recovered to a control level 5–20 min after interruption of infusion of practolol. Dose-response curves for isoprenaline before and during intra-arterial infusions of practolol are shown in Figure 6. In this figure, it should be noted that the response to isoprenaline at a dose as small as 0.03  $\mu\text{g}$  was not completely inhibited by practolol.

**Table 1** Doses (ED, 10 ml/min) of isoprenaline increasing renal blood flow by 10 ml/min in the absence and in the presence of practolol or pindolol and log ED, 10 ml/min ratios in dogs

$\beta$ -Adrenoceptor antagonist	Dose	ED, 10 ml/min ( $\mu\text{g}$ ) (95% confidence limits)	Log ED, 10 ml/min ratio†
Intravenous practolol	0	0.05 (0.02–0.07)	0
	0.3 mg/kg	0.11 (0.07–0.17)*	0.3
	3 mg/kg	0.24 (0.14–0.44)*	0.7
Intra-arterial practolol	0	0.08 (0.06–0.11)	0
	10 $\mu\text{g}/\text{min}$	0.15 (0.12–0.20)*	0.3
	100 $\mu\text{g}/\text{min}$	0.38 (0.29–0.53)*	0.7
	300 $\mu\text{g}/\text{min}$	0.85 (0.45–2.77)*	1.0
Intravenous pindolol	0	0.07 (0.04–0.08)	0
	3 $\mu\text{g}/\text{kg}$	0.59 (0.38–0.95)*	0.9
	10 $\mu\text{g}/\text{kg}$	3.94 (2.23–10.08)*	1.7

† Log ED, 10 ml/min ratio was calculated from the following equation: log ED, 10 ml/min ratio = log ED, 10 ml/min for isoprenaline in the presence of a  $\beta$ -adrenoceptor antagonist minus log ED, 10 ml/min for isoprenaline in the absence of a  $\beta$ -adrenoceptor antagonist.

\* Significantly different from control values ( $P < 0.05$ ).



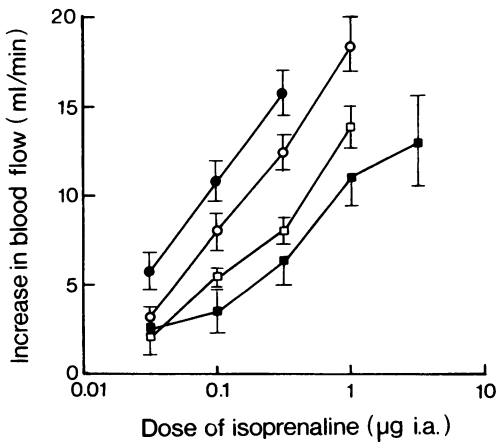
**Figure 5** Effects of isoprenaline injected into the renal artery before and during infusion of practolol into the same artery. Doses of isoprenaline injected into the renal artery were as follows: (a) 0.03  $\mu\text{g}$ ; (b) 0.1  $\mu\text{g}$ ; (c) 0.3  $\mu\text{g}$ ; (d) 1  $\mu\text{g}$ ; (e) 3  $\mu\text{g}$ .

On the other hand, intravenous injections of pindolol (3 and 10  $\mu\text{g}/\text{kg}$ ) shifted the dose-response curves for isoprenaline (0.03–10  $\mu\text{g}$  i.a.) and for salbutamol (0.1–100  $\mu\text{g}$  i.a.) to the right (Figure 7a, b). Log ratios of ED, 10 ml/min for isoprenaline after 3 and 10  $\mu\text{g}/\text{kg}$  i.v. of pindolol to ED, 10 ml/min in

control were 0.9 and 1.7, respectively (Table 1). These values were greater than the log ED, 10 ml/min ratios obtained with 0.3 and 3 mg/kg of intravenous practolol.

**Discussion**

In the present experiments the dose-response curves to isoprenaline and salbutamol for increase in femoral or superior mesenteric blood flow were almost parallel. In both vascular beds salbutamol was approximately 1/15 as potent as isoprenaline on a weight basis. The present results were thus consistent with those obtained by Cullum, Farmer, Jack & Levy (1969) and by Wasserman & Levy (1974) who found that salbutamol was 1/10 to 1/20 as potent as isoprenaline in increasing blood flow through or in decreasing perfusion pressure of the dog hind limb. Salbutamol is more selective on bronchial and vascular  $\beta$ -adrenoceptors than on cardiac  $\beta$ -adrenoceptors (Cullum *et al.*, 1969; Daly, Farmer & Levy, 1971; Wasserman & Levy, 1974) and belongs to selective stimulants of  $\beta_2$ -adrenoceptors classified by Lands *et al.* (1967a, b). In the present experiments the position of the dose-response curve to isoprenaline for increase in femoral blood flow was not significantly altered by intravenous injection of practolol and the dose-response curve to isoprenaline for increase in superior mesenteric blood flow was also not significantly



**Figure 6** Dose-response curves to isoprenaline for increase in renal blood flow before (●) and during infusion of practolol into the renal artery: (○) 10  $\mu\text{g}/\text{min}$ ; (□) 100  $\mu\text{g}/\text{min}$ ; (■) 300  $\mu\text{g}/\text{min}$ . Vertical bars show s.e. mean ( $n = 5$ ).

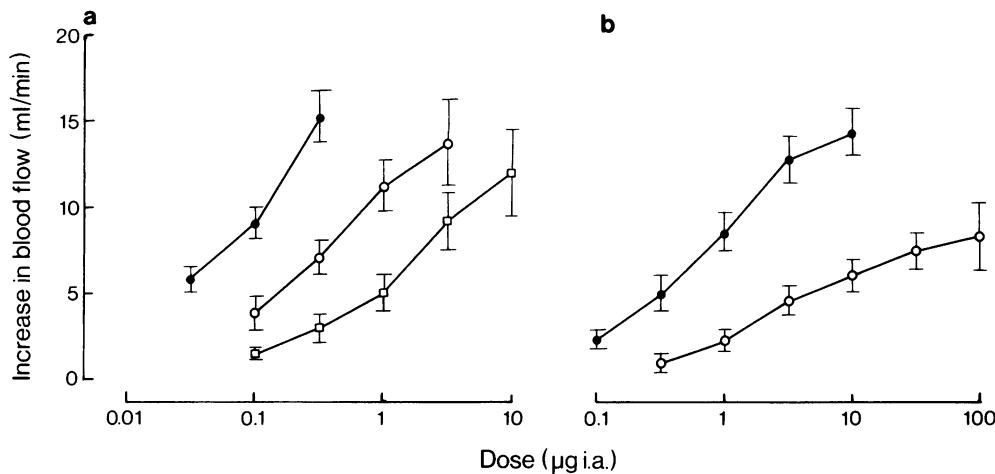


Figure 7 Dose-response curves to (a) isoprenaline and (b) salbutamol for increase in renal blood flow before (●) and after pindolol: (○) 3 μg/kg i.v.; (□) 10 μg/kg i.v. Vertical bars show s.e. mean ( $n=5$ ).

modified by intra-arterial infusion of practolol. In contrast, the two dose-curves for isoprenaline were effectively shifted to the right by pindolol. Practolol antagonizes the positive chronotropic and inotropic effects but not the vasodilator action of isoprenaline (Dunlop & Shanks, 1968), and can be classified as a selective  $\beta_1$ -adrenoceptor antagonist. Unlike practolol, pindolol blocks non-selectively  $\beta$ -adrenoceptors in cardiac muscle and in bronchial and vascular smooth muscle (Giudicelli, Schmitt & Boissier, 1969; Yabuuchi & Kinoshita, 1974). Thus, the present results can be interpreted in such a way that  $\beta$ -adrenoceptors in the femoral and superior mesenteric vascular beds are homogeneous and belong to the  $\beta_2$ -type proposed by Lands *et al.* (1967a,b).

In the present experiments in which the animals were treated initially with phenoxybenzamine, intra-arterial injection of isoprenaline produced an increase in renal blood flow. The results are in accord with those of McNay & Goldberg (1966). Salbutamol also increased the renal blood flow. However, the potency of salbutamol in increasing renal blood flow was approximately 1/240 that of isoprenaline on a weight basis. Thus, the difference in potency between isoprenaline and salbutamol as vasodilators in the renal vascular bed was much greater than that between the two compounds in the femoral or superior mesenteric vascular beds. Furthermore, the dose-response curve to salbutamol for increase in renal blood flow was less steep than that to isoprenaline. These observations suggest that  $\beta$ -adrenoceptors in the renal vascular bed may be different from those in

the femoral and superior mesenteric vascular beds. The dose-response curve to isoprenaline for increase in renal blood flow was shifted almost in a parallel fashion to the right by intravenous or intra-arterial practolol in doses which did not significantly change the dose-response curves to isoprenaline for increases in femoral and superior mesenteric blood flow. However, the renal blood flow response to isoprenaline at a dose as small as 0.03 μg was not completely abolished even by intra-arterial infusion at a maximal rate of 300 μg/min of practolol used in the present experiments. A 10-fold increase in intravenous or intra-arterial doses of practolol produced a shift of approximately 0.4 log units to the right in the dose-response curves to isoprenaline for increase in renal blood flow, whereas only a 3-fold increase in intravenous doses of pindolol induced a shift of 0.8 log units. This difference in displacement of the dose-response curves for isoprenaline by pindolol and practolol may be explained on the basis of the difference in  $\beta$ -adrenoceptor blocking properties of pindolol and practolol, mentioned in the preceding paragraph. Furthermore the increase in renal blood flow induced by salbutamol was not antagonized by practolol but was by pindolol. The failure of practolol to modify the effect of salbutamol may be attributed to the weaker stimulant property of salbutamol on  $\beta_1$ -adrenoceptors than on  $\beta_2$ -adrenoceptors. From these results, it can be concluded that  $\beta$ -adrenoceptors of both the  $\beta_1$ -type and  $\beta_2$ -type proposed by Lands *et al.* (1967a,b) may be demonstrated in the renal vasculature.

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