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ROLE OF 5-HYDROXYTRYPTAMINE IN THE VASOCONSTRICTOR ACTION OF COMPOUND 48/80 IN THE RAT FEMORAL VASCULATURE

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1 The effect of compound 48/80 on the rat femoral vasculature was examined by means of a cross-circulation technique.

2 Intra-arterial injection of increasing doses (1.5 to 15 μg) of compound 48/80 caused dose-dependent vasoconstriction preceded by transient vasodilatation. The vasoconstriction was significantly reduced in reserpine-treated preparations and converted to vasodilatation by methysergide.

3 When the vasoconstrictor response to a large dose (300 μg) of adenosine was abolished by repeated administration, the response to compound 48/80 remained unaltered.

4 The present results indicate that the femoral arterial vasoconstriction by compound 48/80 in the rat is mediated by the release of 5-hydroxytryptamine (5-HT); this 5-HT may be liberated from a different storage site from that released by adenosine.

Introduction

Paton (1951) and Feldberg & Paton (1951) first showed that compound 48/80 had histamine releasing activity. Subsequently this compound has been studied extensively in animals (Johnson & Moran, 1969; Rothshild, 1970; Howland & Spector, 1972). Its specificity as a histamine releaser has created much interest, and compound 48/80 has been used in evaluating the function of the histaminergic system.

However, several workers (Bhattacharya & Lewis, 1956; Moran, Uvnäs & Westerholm, 1962; West, 1963) reported that compound 48/80 liberates not only histamine but also 5-hydroxytryptamine (5-HT) from stores. Recently, it has been suggested that adenosine acts as a potent liberator of 5-HT in the rat femoral vasculature (Sakai & Akima, 1977, 1978; Sakai, 1978). To elucidate further the mode of action of compound 48/80 and to compare it with that of adenosine, the effects of both substances were studied on the femoral vascular bed of the rat.

Methods

Male rats (400 to 500 g) of the Sprague-Dawley strain were anaesthetized with sodium pentobarbitone (65 mg/kg i.p.). The hindlimb preparation has been described in detail (Sakai, 1978; Sakai & Akima, 1978). The completely isolated right hindlimb was perfused with the aid of a peristaltic pump (Mitsumi Science, SJ-1210) at a fixed flow rate through the femoral artery with arterial blood conducted from the carotid artery of a donor (550 to 750 g). The donor was not ventilated artificially. Just before the start of perfusion the animals were given intravenous heparin sodium (Daiichi Kagaku), 1000 units/kg, and 200 units/kg was added to the perfusion circuit at hourly intervals. The mean perfusion pressure was set at a value slightly lower than the mean systemic blood pressure of the donor at the beginning of perfusion with a flow rate of about 3.0 ml/min. Shortly after the start of perfusion the pressure rose slightly, but fell subsequently to reach a new steady-state level. Then, the pressure was re-adjusted to near 100 mmHg and, thereafter, remained almost constant for about 3 h. The perfusion pressure was monitored from a side arm of the perfusion circuit and the systemic blood pressure was measured from the femoral artery of the donor by pressure transducers (Nihon Kohden, MPU-0.5). The perfusion pump was precalibrated and rechecked at the end of the experiment. A square wave electromagnetic flowmeter (Nihon Kohden, MF-25) was used for the measurement of femoral blood inflow. Recordings were made on an ink-writing rectigraph (TOA Electronics, EPR-3T).

Reserpine (Apoplon, Daiichi Seiyaku) was injected subcutaneously in a dose of 5 mg/kg 48 and 24 h for donors, and 72, 48 and 24 h for recipients, respectively, before the experiment. In reserpine-treated preparations, experiments were generally finished within 60 min after the start of perfusion, because

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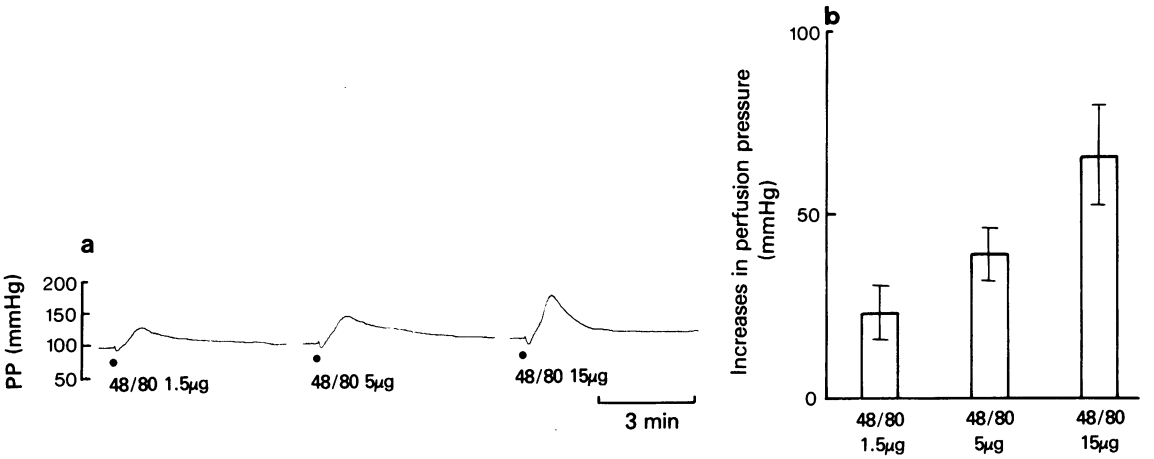

Figure 1 Responses of the femoral vascular bed of rat to increasing doses of compound 48/80. (a) Original tracings. PP, perfusion pressure. (b) Mean data. Vertical bars represent s.e. means from 6 experiments.

the preparations did not remain stable for a long period.

Drugs used were N-methylhomoanisylamine-formaldehyde (compound 48/80, Wellcome Research Labs.), tyramine hydrochloride (Daiichi Kagaku), adenosine and 5-hydroxytryptamine creatinine sulphate (Sigma), (±)-noradrenaline hydrochloride (Sankyo) and methysergide tartrate (Sandoz). The dose of adenosine refers to the base and those of other drugs to their salts. Drugs were freshly prepared with 0.9% w/v NaCl solution (saline) and 0.01 ml of each solution was injected in a period of 4 s into a rubber tube connected to the arterial cannula by means of individual microsyringes (Jintan Terumo Co). Changes in the perfusion pressure caused by drug injections were taken as drug responses, since the perfusion rate was constant.

Values in the text are means ±s.e. mean. The statistical significance of the difference between mean values was analysed with Student's t test and expressed as a P value.

Results

Experiments were performed in 22 untreated and 9 reserpine-treated preparations. The preparations were stable within 20 min of starting perfusion. The basal values of main parameters at this stage are shown in Table 1.

Single injections of compound 48/80 (1.5 to 15 μg) into the femoral artery caused a dose-dependent vasoconstriction preceded by an initial, transient vasodilatation (Figure 1a and b). Intra-arterial injections of

Table 1 Basal values of main parameters in perfused isolated hindlimb of rat

Parameters	*Untreated* (22)	*Reserpine-treated* (9)
Perfusion pressure (mmHg)	100.4 ± 2.2	101.4 ± 2.1
Flow rate (ml/min)	3.6 ± 0.1	3.2 ± 0.3
Femoral vascular resistance $\left(10^4 \times \frac{\text{dyne}\cdot\text{s}}{\text{cm}^5}\right)$	224.4 ± 11.2	264.1 ± 30.1
Systemic blood pressure of the donor (mmHg)	118.5 ± 6.4	68.0 ± 2.6*

The preparations became stable within 20 min after the start of perfusion. At this stage, each value (mean ± s.e. mean) was obtained. The numbers of experiments are given in parentheses.

*$P < 0.01$ compared with the untreated group.

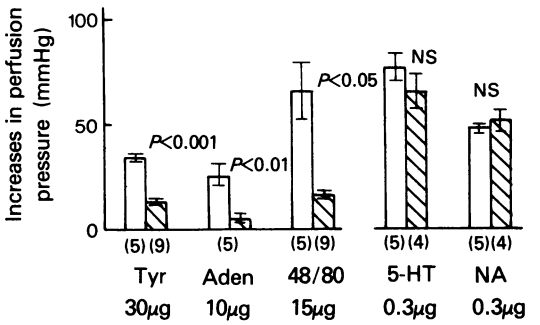

Figure 2 Responses of the femoral vascular bed to tyramine (Tyr), adenosine (Aden), compound 48/80, 5-hydroxytryptamine (5-HT) and noradrenaline (NA) in untreated and reserpine-treated preparations. Open columns: untreated; hatched columns: treated with reserpine (subcutaneously in a dose of 5 mg/kg 48 and 24 h for donor rats, and 72, 48 and 24 h for recipients, respectively, before the experiment). Vertical bars represent s.e. mean, number of experiments are given in parentheses. NS, not significant.

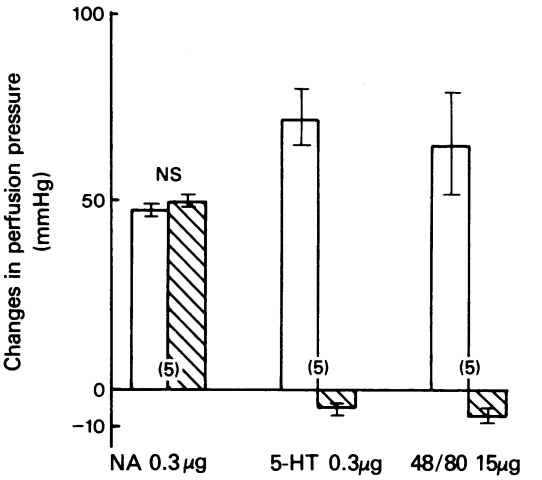

Figure 3 Effects of methysergide on the vasoconstrictor responses to noradrenaline (NA), 5-hydroxytryptamine (5-HT) and compound 48/80. Open columns: untreated; hatched columns: treated with methysergide (1 μg). Vertical bars represent s.e. mean, number of experiments are given in parentheses. NS: not significant.

these doses of 48/80 had no effect on the systemic blood pressure of the donor rats. The effects of reserpine pretreatment on responses to tyramine (30 μg), adenosine (10 μg), 48/80 (15 μg), 5-HT (0.3 μg) and noradrenaline (0.3 μg) are shown in Figure 2. Reserpine-induced depletion of endogenous 5-HT and catecholamines did not alter vascular responses to exogenously administered 5-HT and noradrenaline in contrast to the vasoconstrictor responses to tyramine, adenosine and 48/80, which were significantly reduced.

The effect of methysergide was examined on the vasoconstrictor response to compound 48/80. Since tachyphylaxis developed to the repeated administration of 48/80, only a single dose (15 μg) was used in one preparation. In untreated preparations, 5-HT (0.3 μg), 48/80 (15 μg) and noradrenaline (0.3 μg) were given in that order and responses to these substances served as controls. In methysergide-treated preparations 5-HT and noradrenaline were given before methysergide and 5-HT, 48/80 and noradrenaline were injected after methysergide in that order. As shown in Figure 3, an injection of methysergide (1 μg) which did not affect the perfusion pressure, prevented the response to 5-HT, but did not abolish that to noradrenaline. The same dose of methysergide converted the vasoconstrictor response to 48/80 to vasodilatation.

The vasoconstrictor effect of compound 48/80 (15 μg) after the repeated administrations of a large dose (300 μg) of adenosine was examined on this vascular bed. The first injection of adenosine (300 μg) caused a marked and long-lasting increase in the perfusion pressure (92.8 ± 17.5 mmHg, $n = 5$). Compound 48/80 was not injected before adenosine, because the repeated administration of 15 μg of 48/80 produced a definite tachyphylaxis. Upon 4 consecutive injections of the same dose (300 μg) of adenosine, the rise in the perfusion pressure (vasoconstriction) was reduced progressively, and finally it almost disappeared (6.2 ± 1.4 mmHg, $n = 5$). At this point, the vasoconstrictor response to 48/80 (15 μg) was not significantly reduced in comparison with the control response obtained from other preparations (Figure 1) (control, 59.5 ± 12.8 mmHg, $n = 6$; after adenosine, 31.2 ± 5.5 mmHg, $n = 5$; $P > 0.05$).

Discussion

The present experiments revealed that when injected into the femoral artery, compound 48/80 caused a considerable vasoconstriction. This vascular response required investigation, since compound 48/80 is generally known as a potent histamine liberator (Paton, 1951; Feldberg & Paton, 1951; Johnson & Moran, 1969; Rothschild, 1970; Howland & Spector, 1972) and histamine causes vasodilatation in the present preparation (Sakai, unpublished data).

Since the vasoconstriction in response to compound 48/80 appeared significantly less in reserpine-treated preparations compared with that in untreated ones, catecholamines or 5-HT were considered as possible mediators (Shore, 1962).

The vasoconstrictor responses to compound 48/80 as well as 5-HT and adenosine were completely blocked after methysergide, but the response to noradrenaline was not prevented. This would indicate that compound 48/80 releases 5-HT in the rat hindlimb and that the induced-vasoconstriction is mediated by a tryptaminergic mechanism. This finding and conclusion is supported by previous reports that compound 48/80 liberates 5-HT as well as histamine from the mast cells of rats (Bhattacharya & Lewis, 1956; Moran *et al.*, 1962; West, 1963). The finding that adenosine is a possible 5-HT liberator in the rat femoral vasculature (Sakai, 1978; Sakai & Akima, 1977, 1978) lends further support.

However, even when the vasoconstrictor response to adenosine had disappeared after a series of consecutive doses, the response to compound 48/80 injected into the femoral artery remained unaltered. It is likely, therefore, that compound 48/80 liberates 5-HT from a different storage site from that released by adenosine.

The present results suggest that compound 48/80 induced a prominent vasoconstriction of the femoral vascular bed of the rat resulting from an indirect action by the release of 5-HT from stores.

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