The role of α_2 -adrenoceptors in the vasculature of the rat tail

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1 The effects of α_2 -adrenoceptor agonists and antagonists on rat tail skin temperature (t_{is}), an indicator of local cutaneous blood flow, were studied in conscious and anaesthetized rats and in the isolated, Krebs perfused, vascular bed of the rat tail.

2 In conscious rats, at an ambient temperature of 18.5–20°C, t_{is} was $21.0 \pm 0.2^\circ\text{C}$ and core (rectal) temperature (t_c) was $38.2 \pm 0.04^\circ\text{C}$ ($n = 126$). The α_2 -adrenoceptor antagonist, delequamine (RS-15385-197; 1 mg kg^{-1} , s.c., $n = 6$), produced a rapid elevation in t_{is} to $29.1 \pm 0.7^\circ\text{C}$ ($P < 0.001$ vs. saline-treated control group), attained 10 min after injection. t_c fell slightly, by $1.0 \pm 0.1^\circ\text{C}$. The t_{is} response was dose-related over the dose-range tested (0.01 – 1 mg kg^{-1} , s.c.), with an ED_{50} of $17 \mu\text{g kg}^{-1}$, s.c. ($n = 6$ per dose).

3 The maximum increases in t_{is} in response to a dose of 1 mg kg^{-1} , s.c. of α_2 -adrenoceptor antagonists were as follows ($n = 6$ per drug): delequamine ($+ 9.6 \pm 0.8^\circ\text{C}$) > yohimbine ($+ 9.0 \pm 1.0^\circ\text{C}$) > WY-26703 ($+ 7.9 \pm 1.3^\circ\text{C}$) > piperoxan ($+ 5.6 \pm 1.7^\circ\text{C}$) > idazoxan ($+ 4.6 \pm 1.3^\circ\text{C}$) > imiloxan ($+ 4.1 \pm 1.3^\circ\text{C}$) > SKF 104078 ($+ 2.0 \pm 1.9^\circ\text{C}$) > BDF-6143 ($+ 1.3 \pm 0.8^\circ\text{C}$).

4 Prazosin (0.3 mg kg^{-1} , s.c.), hydralazine (10 mg kg^{-1} , s.c.) and nifedipine (3 mg kg^{-1} , s.c.) did not increase t_{is} , whereas propranolol (10 mg kg^{-1} , s.c.) evoked a small increase in t_{is} ($+ 2.9 \pm 1.0^\circ\text{C}$). Pentolinium (2 – 10 mg kg^{-1} , s.c.) elicited a dose-related increase in t_{is} , which was elevated by $4.4 \pm 1.3^\circ\text{C}$ after a dose of 10 mg kg^{-1} ; t_c was reduced in a dose-related manner. Drug vehicles (1 ml kg^{-1} , s.c.) had no effect on t_{is} or t_c .

5 In anaesthetized rats, idazoxan ($300 \mu\text{g}$, i.v.) produced a rapid increase in t_{is} which was detectable 2 min after beginning the injection, reaching a peak after 7 min. When the same dose was administered i.c.v., t_{is} also rose, but more slowly. The peak response ($+ 3.6 \pm 0.7^\circ\text{C}$, $n = 5$) was significantly smaller than when idazoxan was administered intravenously ($+ 6.3 \pm 1.2^\circ\text{C}$, $n = 5$), which suggests that the increase in t_{is} following systemic administration of α_2 -adrenoceptor antagonists is not due to a central effect. The change in t_{is} was not secondary to changes in blood pressure.

6 In the isolated, Krebs perfused, tail vascular bed of the rat, at an ambient temperature of 20–21°C, under constant flow conditions (3.5 – 4.0 ml min^{-1} ; $n = 4$), baseline perfusion pressure was $57 \pm 4 \text{ mmHg}$. 5-Hydroxytryptamine (5-HT; 70 – 150 nM) increased perfusion pressure by $56 \pm 9 \text{ mmHg}$. The α_2 -adrenoceptor agonist, UK-14,304 (10 nmol), elicited a further increase in perfusion pressure by $27.5 \pm 15 \text{ mmHg}$ but had no effect in the absence of 5-HT; this response to UK-14,304 was abolished by rauwolsine ($1 \mu\text{M}$).

7 Under constant pressure conditions ($\sim 100 \text{ mmHg}$; $n = 9$), baseline mean perfusion flow was $2.1 \pm 0.2 \text{ ml min}^{-1}$, and mean tail skin temperature was $31.6 \pm 0.6^\circ\text{C}$. 5-HT ($119 \pm 28 \text{ nM}$) decreased t_{is} by $3.3 \pm 2.0^\circ\text{C}$ and reduced flow by $1.2 \pm 0.3 \text{ ml min}^{-1}$. UK-14,304 (10 nmol) further reduced t_{is} by $3.0 \pm 0.3^\circ\text{C}$ without significant effect on flow; this effect was also abolished by $1 \mu\text{M}$ rauwolsine.

8 We conclude that post-junctional α_2 -adrenoceptors in the vasculature of the rat tail have a major vasoconstrictor role, controlling both the flow and distribution of blood within the tail and thereby thermoregulatory heat loss from its surface.

Keywords: α_2 -Adrenoceptors; rat tail; thermoregulation; cutaneous vasoconstriction; delequamine; RS-15385-197

Introduction

The highly vascularized tail of the rat is an important organ of thermoregulation in this species (Rand *et al.*, 1965). The resting temperature of the tail skin is normally only a degree or so above the ambient air temperature, but if body core temperature rises above a critical threshold (approximately 39°C ; Thomson & Stevenson, 1965; Little & Stoner, 1968), the tail skin temperature is rapidly elevated to within 3 – 4°C of the body core temperature (Grant, 1963; O'Leary *et al.*, 1985), and up to 20% of the total heat production of the rat may thereby be dissipated via the tail (Rand *et al.*, 1965). This is indicative of an active process of thermoregulatory heat loss, and is known to be under the control of the

hypothalamus (Thomson & Stevenson, 1965; Young & Dawson, 1982). The most comprehensive study of the vascularity of rodent tails was carried out by Thorington (1966), who noted many similarities to other mammalian extremities, including human digits, particularly in the complex arrangement of both arterial and venous shunts. Thus, the increase in tail skin temperature during thermoregulatory heat loss could be brought about by generalized vasodilatation of arterial and/or venous vessels within this organ, preferential vasodilatation of surface vessels, redistribution of blood flow from core vessels to surface vessels by the opening of shunt vessels, or opening of arterio-venous anastomoses within the tail.

The pharmacological basis of the control of blood flow through and within the rat tail is unclear. In intact, conscious

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rats, a variety of systemically-administered agents have been shown to raise the tail skin temperature, usually in studies on central neurotransmitters involved in thermoregulation (e.g. Cox & Lee, 1977). Few of these studies addressed the question of the local pharmacology of the rat tail vasculature. During *in vivo* testing of the potent, selective α_2 -adrenoceptor antagonist, delequamine (RS-15385-197; Brown *et al.*, 1993; Redfern *et al.*, 1993), we casually observed an apparent warming of the tail following its administration to conscious rats. We therefore set out to study this phenomenon more systematically, addressing such questions as to whether it was a general feature of α_2 -adrenoceptor antagonists and whether this was a local or central effect.

There are few examples *in vitro* of isolated blood vessels that possess a functional population of post-junctional α_2 -adrenoceptors. In the isolated vascular bed of the rat tail, however, it has been shown that raising the vascular tone with vasopressin or endothelin can uncover responses to the α_2 -adrenoceptor agonist, UK-14,304 (Templeton *et al.*, 1989; MacLean & McGrath, 1990). This preparation was, therefore, modified to study the effects of UK-14,304 on the skin temperature of the rat isolated tail. 5-Hydroxytryptamine (5-HT) was used to raise the basal vascular tone.

Preliminary accounts of part of this work have been presented to the British Pharmacological Society (Redfern & Clague, 1991; Redfern *et al.*, 1994).

Methods

Studies in unanaesthetized rats

Male Sprague-Dawley rats weighing 200–330 g were used. They were housed in pairs in standard wire cages at an ambient temperature of 18–20°C from arrival in the animal unit 2–6 days before the experiment. The experiments were conducted between 10 h 00 min–16 h 00 min at an ambient temperature of 18.5–20.0°C. The rats were placed in well-ventilated perspex holders (length 18 cm, width 12 cm, height 10 cm) and lightly restrained by passing the tail through an aperture at the rear of the cage and taping it to a perspex rod which protruded from the rear wall above the opening. Rectal core temperature was monitored by a thermocouple which was inserted 5 cm past the anal sphincter and taped to the tail. A flat disc thermocouple (diameter 7 mm; RS Components Ltd) was affixed to the dorsal skin of the tail, 7 cm from the tip, using two layers of surgical tape (Elastoplast fabric tape; Smith & Nephew Ltd). The rats were able to move their head limbs easily, and usually became settled within a few minutes. Readings of ambient temperature (t_a), rectal core temperature (t_c) and dorsal tail skin temperature (t_{ts}) were taken at 5 min intervals for 75 min, beginning 60 min after first placing the rats in the holders. Immediately after the fourth reading the rats received drug or vehicle by subcutaneous injection (1 ml kg⁻¹) into the nape of the neck.

Studies in anaesthetized rats

Male Sprague-Dawley rats weighing 260–330 g were anaesthetized with sodium pentobarbitone (60 mg kg⁻¹, i.p.). Core temperature was monitored from the rectum and maintained at 36–38°C with a heating lamp. Cannulae were inserted in the trachea, to facilitate respiration, the left jugular vein, to permit injections of drug and supplementary injections of anaesthetic, and the left carotid artery, to record arterial blood pressure. Recordings of blood pressure and heart rate were displayed on a Lectromed Multitrace 4 chart recorder. Tail skin temperature was measured from the dorsal surface 7 cm from the tip as described above. The thermocouple was connected to a custom-built thermocouple amplifier, and the measurement displayed on a Lectromed Multitrace 4 chart recorder. The tail was shielded from the heating lamp by a

sheet of aluminium foil. Air temperature beneath the shield was monitored close to the tail by means of a thermometer, and remained within the range 22.5 to 25°C; within any individual experiment it remained within 1°C of the pre-drug value.

I.c.v. injections The head was fixed in a stereotaxic frame in the orientation of Paxinos & Watson's (1982) atlas. The skull was exposed and cleared of connective tissue, and a small burr-hole drilled 1.6 mm lateral to the mid-line and 0.8 mm caudal to bregma. The injection system consisted of a 30G steel injection cannula connected via a length of polythene cannula to a 50 μ l Hamilton syringe; the injection cannula was held inside a 22G guide cannula mounted in a micro-manipulator, such that the tip of the injection cannula protruded 1.5 mm beyond the tip of the guide cannula. The injection assembly was lowered into the brain so that the tip of the injection cannula was 3 mm below the brain surface. When the records had stabilized, a single injection of idazoxan (300 μ g) was made in a volume of 10 μ l injected over 60 s. After completing the experiment the location of the cannula tip in the ventricle was confirmed by injection of 10 μ l Indian ink. In each case, the ink was distributed throughout the ventricular system as far as the spinal canal.

I.v. injection The rat was placed in a prone position with its head raised by supporting the upper incisors, to facilitate respiration. When the records had stabilized, a single injection of idazoxan (300 μ g) was made in a volume of 0.05 ml flushed in over 60 s with 0.1 ml saline. The cannula dead space was 0.05 ml. The responses were followed over 1 h.

In vitro studies

Male Wistar rats weighing 275–325 g were killed by stunning followed by cervical dislocation. The ventral surface of the tail was shaved at the base before amputation by cutting through an intervertebral disc. The skin was reflected around the proximal first 3–5 cm of the tail, and a longitudinal incision made through the midline of the fascia on the underside of the tail. The tail artery was exposed using blunt forceps and cleaned of fat and connective tissue. Following insertion of a polyethylene cannula (o.d. 0.75 mm) into the tail artery, the tail was placed on a perspex platform and the arterial cannula attached to a perfusion circuit. The tail was perfused with modified physiological salt solution (gassed with 95% O₂: 5% CO₂), the temperature of which was altered to ensure that the perfusate entering the tail was at 37°C (measured with a thermocouple (RS Components Ltd)). The ambient temperature was 20–21°C.

Constant flow experiments Flow was held at 3.5–4.0 ml min⁻¹. After the start of perfusion, blood filled drops were observed from the cut end of the tail, indicating a successful cannulation. A flat disc thermocouple (diameter 7 mm; RS Components Ltd) linked to a digital thermometer (Digitron Instrumentation Ltd) was taped to the dorsal surface of the rat tail some 13–16 cm from the tip, just below the point of cannulation. This was connected to a flat-bed recorder to give continuous temperature measurements. In one preparation, after setting up under constant flow conditions, t_{ts} was measured with the flat disc thermocouple, at 1 cm distances from the tip of the tail.

Constant pressure experiments A reservoir, open to the pressure transducer, was situated on a shelf at a fixed distance above the tail to give a constant pressure of approximately 100 mmHg. Blood flow out of the tail was determined by collection of perfusate into a measuring cylinder. The temperature of the perfusate leaving the tail was also measured with the thermocouple.

Experiments were initially conducted under constant flow

conditions to investigate the effect of 5-HT and UK-14,304 on perfusion pressure and then the preparation was set up under constant pressure. UK-14,304 (10 nmol) was administered as a 10 μ l bolus injection into an injection port situated just prior to the inflow cannula. 5-HT and rauwols-cine were included in the perfusate at the required concentration.

Statistical analysis

For the *in vivo* studies, differences between groups were tested by repeated measures analysis of variance; if there was a significant interaction between treatments over time, the groups were compared at each time point with two-tailed *t* test (using the residual variance). For *in vitro* studies, differences between groups were tested with a two-tailed *t* test.

Drugs used

Pentobarbitone sodium ('Sagatal') and pentolinium tartrate (M & B); clonidine HCl, guanethidine sulphate, hydralazine, nifedipine, prazosin HCl and yohimbine HCl (Sigma); 5-hydroxytryptamine creatinine sulphate (BDH biochemicals); UK-14,304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline bitartrate (Pfizer); rauwols-cine HCl (Roth); BDF 6143 (4-chloro-2-[2-imidazolin-2-ylamino]-isoindoline hydrochloride), dipropyl 5-carboxamido tryptamine (DP5-CT), idazoxan HCl, imiloxan HCl, piperoxan HCl, delequamine (RS-15385-197), SKF 104078 (6-chloro-9-(3-methyl-2-butenyl)oxyl-3-methyl-1*H*-2,3,4,5-tetrahydro-3-benzazepine maleate) and WY26703 (N-methyl-N-(1,3,4,6,7,11 β -hexahydro-2*H*-benzo(a)-quinolizin-2 β -yl) isobutane sulphonamide) were synthesized by Dr R. Clark, Syntex, Palo Alto, California, U.S.A. In the studies in anaesthetized rats, idazoxan HCl was dissolved in saline at a concentration of 6 mg (base) ml⁻¹ (i.v. dosing) or 30 mg (base) ml⁻¹ (i.c.v. administration); higher concentrations could not be achieved.

In the *in vitro* studies, the composition of the physiological salt solution was (in mM): NaCl 118.4, NaHCO₃ 25, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1.2, CaCl₂ 2.5, glucose 11, Na₂EDTA 0.023. Ficoll 2% (molecular weight approximately 70,000) was included to prevent excessive oedema within the tail.

Results

Unanaesthetized rats

In conscious rats, lightly restrained at an ambient temperature of 18.5–20°C, *t*_{is} was 21.0 ± 0.2°C and core (rectal) temperature (*t*_c) was 38.2 ± 0.04°C (*n* = 126).

α_2 -Adrenoceptor antagonist At a dose of 1 mg kg⁻¹, s.c., delequamine (RS-15385-197) evoked a rapid and prolonged increase in *t*_{is} to a peak of 29.1 ± 0.7°C (*n* = 6) with a small (<1°C) decrease in *t*_c (Figure 1). The threshold dose for evoking an increase in *t*_{is} was 0.01 mg kg⁻¹, s.c. Yohimbine (1 mg kg⁻¹, s.c.) and idazoxan (1 mg kg⁻¹, s.c.) also produced a rapid increase in *t*_{is} (Figure 2); however, the response to idazoxan (1 mg kg⁻¹, s.c.) was only slightly larger than that evoked by a low dose of delequamine (0.01 mg kg⁻¹, s.c.). The order of apparent potency for the α_2 -adrenoceptor antagonists in elevating tail skin temperature at a dose of 1 mg kg⁻¹, s.c., was as follows (peak increases in *t*_{is} in parentheses; *n* = 6 for each): delequamine (+9.6 ± 0.8°C) > yohimbine (+9.0 ± 1.0°C) > WY-26703 (+7.9 ± 1.3°C) > piperoxan (+5.6 ± 1.7°C) > idazoxan (+4.6 ± 1.3°C) > imiloxan (+4.1 ± 1.3°C) > SKF 104078 (+2.0 ± 1.9°C) > BDF-6143 (+1.3 ± 0.8°C).

Other agents (*n* = 6 for each) The α_2 -adrenoceptor agonist, clonidine (0.3 mg kg⁻¹, s.c.), produced a small, transient in-

Figure 1 Effects of the selective α_2 -adrenoceptor antagonist, delequamine, on core (rectal) temperature and dorsal tail skin temperature (measured 7 cm from the tip) in conscious, lightly restrained rats. (○), Saline 1 ml kg⁻¹, s.c.; (●) delequamine at doses of (a) 0.01 mg kg⁻¹, s.c.; (b) 0.1 mg kg⁻¹, s.c.; (c) 1 mg kg⁻¹, s.c. Injection at arrow. Values are mean ± s.e.mean, *n* = 6. **P* < 0.05; ***P* < 0.01, drug-treated vs. vehicle-treated group, *t* test at each point using residual variance following repeated measures ANOVA.

Figure 2 Maximal increases in tail skin temperature evoked by a range of α_2 -adrenoceptor antagonists administered to conscious rats at a dose of 1 mg kg⁻¹, s.c. (*n* = 6 per group). The data are the mean of maximal increases in *t*_{is} (maximum *t*_{is} attained following drug administration (regardless of latency) minus *t*_{is} immediately before drug administration) for each individual within a treatment group.

Figure 3 Effects of the ganglion blocking agent, pentolinium, on *t*_c and *t*_{is} in conscious rats. (○), Saline 1 ml kg⁻¹, s.c.; (●) pentolinium at doses of (a) 2 mg kg⁻¹, s.c.; (b) 5 mg kg⁻¹, s.c.; (c) 10 mg kg⁻¹, s.c. Injection at arrow. Values are mean ± s.e.mean, *n* = 6. **P* < 0.05; ***P* < 0.01; ****P* < 0.001, drug-treated vs. vehicle-treated group, *t* test at each time point using residual variance following repeated measures ANOVA. In (b), there was a significant overall treatment effect on *t*_{is} averaged over time (*P* = 0.0387); in (c) there was a significant interaction between treatment and time in the *t*_{is} data (*P* = 0.204). *Post-hoc t* tests did not reveal any significant differences in *t*_{is} for any individual time point, for any of the three doses.

crease in t_{is} ($+1.9 \pm 0.6^\circ\text{C}$) accompanied by a large decrease in t_c and piloerection. The ganglion blocking agent pentolinium (2 and 5 mg kg $^{-1}$, s.c.) evoked a small increase in t_{is} and a 2°C decrease in t_{is} (Figure 3). At a higher dose (10 mg kg $^{-1}$, s.c.) pentolinium elevated t_{is} by $4.4 \pm 1.3^\circ\text{C}$, with a concomitant decrease in core temperature ($-3.8 \pm 0.8^\circ\text{C}$; Figure 3). The α_1 -adrenoceptor antagonist, prazosin (0.3 mg kg $^{-1}$, s.c.; Figure 4) and the adrenergic neurone blocking agent, guanethidine (10 mg kg $^{-1}$, s.c.), had no effect on t_{is} or t_c . The β -adrenoceptor antagonist, propranolol (10 mg kg $^{-1}$, s.c.), produced a small increase in tail skin temperature ($+2.0 \pm 1.0^\circ\text{C}$) accompanied by a small ($\sim 1^\circ\text{C}$) fall in core temperature (Figure 4). The vasodilator agent, hydralazine (10 mg kg $^{-1}$, s.c.), lowered t_c (-3°C), which was delayed in onset by some 20 min, but did not affect t_{is} , whereas the calcium antagonist, nifedipine (3 mg kg $^{-1}$, s.c.), was inactive. The 5-HT $_{1A}$ receptor agonist, dipropyl 5-carboxamido tryptamine (DP5-CT; 1 mg kg $^{-1}$, s.c.), produced a large decrease in t_c (-4°C) without changing t_{is} . The injection of an equivalent volume of water or saline (1 ml kg $^{-1}$, s.c.) had no effect on either parameter.

Anaesthetized rats

Tail skin temperature The tail skin temperature (t_{is}) of each animal was very stable ($23.3 \pm 0.2^\circ\text{C}$; $n = 10$) until administration of idazoxan. Whereas blood pressure fluctuated before drug administration, this had no effect on t_{is} . Core temperature began to fall after injection of idazoxan, but this was arrested by use of the heating lamp, and was maintained within 1°C of the pre-drug value. Idazoxan (300 μg , i.v.) produced a rapid increase in t_{is} which was detectable 2 min after beginning the injection, reaching a peak 7 min after beginning the injection (Figure 5a). When the same dose was administered i.c.v., t_{is} also rose, but more slowly: no detectable effect was seen until 4 min after injection, and the response reached a peak 10 min after injection (Figure 5a). Also, the peak response ($+3.6 \pm 0.7^\circ\text{C}$; $n = 5$) was substantially smaller than when idazoxan was administered intravenously ($+6.3 \pm 1.2^\circ\text{C}$; $n = 5$). Statistically significant differences between the responses elicited by the two routes of administration were seen from 3–15 min after injection (Figure 5a).

Mean arterial blood pressure Before injection of idazoxan, mean arterial blood pressure in this group of rats was 118.4 ± 7.2 mmHg ($n = 10$). Changes in blood pressure which followed the administration of idazoxan were small but complex. After the i.v. dose, there appeared to be a biphasic response: MAP appeared to fall slightly (by 6.5 ± 7.3 mmHg)

during the injection and then rose, reaching a peak ($+8.5 \pm 2.3$ mmHg) 1.5 min after beginning the injection. With i.c.v. injection, there was no change in MAP during the injection period, but MAP rose gradually by $3.9 \pm 2.3^\circ\text{C}$ (6 min after beginning the injection). MAP then fell below the pre-drug level by 7.3 ± 1.7 mmHg (Figure 5b). With either route of administration, the changes in t_{is} did not merely follow the changes in blood pressure.

Heart rate Before injection of idazoxan, heart rate in this group of rats was 397.6 ± 12.7 b.p.m. ($n = 10$). Upon i.v. administration of idazoxan there was an immediate tachycar-

Figure 5 Comparison of effects of i.v. (○) and i.c.v. administration (●) of the α_2 -adrenoceptor antagonist, idazoxan (300 μg), on (a) tail skin temperature, (b) mean arterial blood pressure and (c) heart rate, in pentobarbitone-anaesthetized rats. Values are mean $\pm$ s.e.mean, $n = 5$. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, i.v. vs. i.c.v. treated group, t test at each time point using residual variance following repeated measures ANOVA.

Figure 4 Effects of (a) the selective α_1 -adrenoceptor antagonist, prazosin (0.3 mg kg $^{-1}$, s.c.), and (b) the selective β -adrenoceptor antagonist, propranolol (10 mg kg $^{-1}$, s.c.) on t_c and t_{is} in conscious, lightly restrained rats. Values are mean $\pm$ s.e.mean, $n = 6$. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, drug-treated vs. vehicle-treated group t test at each time point using residual variance following repeated measures ANOVA.

Figure 6 Dorsal skin temperatures at different points along an isolated tail of rat perfused with Krebs solution at constant flow (0.8 ml min $^{-1}$), with perfusate entering the base of the tail at 37°C . The ambient temperature was 20°C .

dia which began within seconds of commencing the 60 s injection, reaching a peak of $+41.8 \pm 4.0$ b.p.m. at the end of the 1 min injection period, and descending to the pre-drug level 6 min after injection. Heart rate then rose again, and was still elevated by some 35–40 b.p.m. 60 min after injection. Following i.c.v. injection there was also a very rapid tachycardia. However, this did not reach the same peak although it was sustained for about 10 min. There was no secondary increase in heart rate (Figure 5c).

In vitro studies

Under constant flow conditions ($3.5\text{--}4.0$ ml min⁻¹), perfusion pressure was 57 ± 4 mmHg ($n = 4$). In each preparation, we established a concentration of 5-HT which approximately doubled perfusion pressure. In four preparations, 5-HT (119 ± 28 nM) raised perfusion pressure by 56 ± 9 mmHg. A bolus injection of UK-14,304 (10 nmol in 10 μ l) caused an increase of perfusion pressure of 27.5 ± 15 mmHg in the presence of 5-HT but did not have an effect in its absence. Inclusion of 1 μ M rauwolscine in the perfusate abolished responses to UK-14,304 ($P < 0.05$). Figure 6 shows the rat tail skin temperature at 1 cm distances from the tip of the tail under constant flow conditions. It shows that, in this preparation, the t_{18} was reduced by $\sim 14^\circ\text{C}$ from the base of the tail to the tip.

In nine preparations, under constant pressure conditions, the temperature of the perfusate entering the tail was $37.0 \pm 0.4^\circ\text{C}$ and the temperature of the perfusate leaving the tail was $26.5 \pm 0.3^\circ\text{C}$. The fixed perfusion pressure was 99.4 ± 3.5 mmHg, the skin temperature was $31.6 \pm 0.6^\circ\text{C}$ and mean perfusate flow through the tail was 2.1 ± 0.2 ml min⁻¹. 5-HT (119 ± 28 nM) decreased t_{18} by $3.3 \pm 2^\circ\text{C}$ and reduced flow by 1.2 ± 0.3 ml min⁻¹ ($n = 4$). Under these conditions, UK-14,304 (10 nmol in 10 μ l) further reduced t_{18} by $3.0 \pm 0.3^\circ\text{C}$ but flow was not significantly altered, increasing by 0.6 ± 0.5 ml min⁻¹ ($n = 4$). The effects of UK-14,304 were abolished by inclusion of 1 μ M rauwolscine in the perfusate ($P < 0.05$).

Discussion

In the present study, a variety of α_2 -adrenoceptor antagonists were shown to elevate tail skin temperature in the conscious rat. This was not secondary to an increase in core temperature, as this actually fell slightly. Apart from an incidental observation by Wilson & Fregly (1985) relating to yohimbine, this is the first systematic study of this phenomenon. Several questions arose following our initial observations: was the increase in tail skin temperature due to blockade of α_2 -adrenoceptors *per se*; was it unique to blockade of α_2 -adrenoceptors; was it a centrally, systemically or locally-mediated effect; was it secondary to changes in blood pressure; was it merely part of a generalized vasodilatation; and was it brought about by generalized vasodilatation of arterial and/or venous vessels within this organ, preferential vasodilatation of superficial vessels, shunting of blood flow from core vessels to surface vessels, or opening of arterio-venous anastomoses within the tail?

The increase in t_{18} seen with dequalamine (RS-15385-197) was most probably related to α_2 -adrenoceptor blockade as it was also evoked by a range of other α_2 -adrenoceptor antagonists. The putative receptor involved appears to be an α_2 -adrenoceptor rather than an imidazoline receptor, as the response was also evoked not only by idazoxan but also by dequalamine and yohimbine, which, unlike idazoxan, do not interact with imidazoline binding sites (Brown *et al.*, 1990; 1993). However, the α_2 -adrenoceptor antagonists tested at a dose of 1 mg kg⁻¹ for effects on tail skin temperature produced a wide range of magnitude of response. This was poorly correlated with their i.v. ED₅₀ either for peripheral (UK-14,304 pressor responses in pithed rats) or central (UK-

14,304 mydriasis) α_2 -adrenoceptor antagonism (Brown *et al.*, 1993). These data may hint at the involvement of an α_2 -adrenoceptor sub-type, or differential selectivity for pre-junctional α_2 -adrenoceptors, in that increased transmitter release could compensate for blockade of post-junctional α_2 -adrenoceptors. Alternatively, this broad range of response could be a result of differences in absorption from the subcutaneous injection site.

The question of whether the increase in t_{18} was centrally mediated was addressed by comparing responses to the same dose of idazoxan (300 μ g) given either by intravenous or intracerebroventricular injection, in anaesthetized rats. This agent elicited an increase in tail skin temperature by both routes of administration. However, following i.c.v. injection the response was both of a smaller magnitude and delayed in onset. Drugs administered into a lateral ventricle diffuse to distant brain regions, but are also absorbed into the general circulation. Thus, our data indicate either that the response is elicited from a site distant from the ventricles or else the drug is diffusing out of the brain to effect its response from a peripheral site. If the former were true it would be expected that the response would be of the same magnitude, if not larger, than that elicited by the same dose given intravenously. As this was not the case, absorption into the general circulation seems a more likely explanation. The blood pressure responses were different following the two routes of administration, but in both cases were small and complex. It is, however, worth noting that in neither case did the change in tail skin temperature follow the change in blood pressure. The initial tachycardia was immediate by either route, suggesting that idazoxan given centrally elicited tachycardia by a central action, and confirming that the i.c.v. injections were successful. These experiments with idazoxan pointed to a peripheral site of action for the α_2 -adrenoceptor antagonists in elevating tail skin temperature. The suggestion that α_2 -adrenoceptors located in the tail vasculature were responsible for such thermoregulatory changes was confirmed by the results of the *in vitro* studies. These data showed a direct cooling effect of an α_2 -adrenoceptor agonist in the rat isolated tail. This effect was inhibited by a selective α_2 -adrenoceptor antagonist, verifying that the response was α_2 -adrenoceptor-mediated. Prior to introduction of 5-HT and UK-14,304, the temperature of the perfusate was decreased by more than 10°C on passage through the tail, indicating that the vasculature was markedly dilated.

The most straightforward hypothesis to emerge from these studies was that the α_2 -adrenoceptors involved were located post-junctionally within the tail vasculature, and were responsible for mediating tonic sympathetic vasoconstriction within this vascular bed. Therefore, interruption of sympathetic vasoconstrictor tone with a ganglion blocking agent would be expected to result in responses of a similar magnitude to those elicited by antagonists at α_2 -adrenoceptors. The ganglion-blocking agent, pentolinium (10 mg kg⁻¹, s.c.), elevated t_{18} by 4.4°C , which was greater than the response at 5 mg kg⁻¹ ($+3.4^\circ\text{C}$), but was only approximately 45% of the largest response evoked by an α_2 -adrenoceptor antagonist ($+9.6^\circ\text{C}$, dequalamine). However, core temperature (and therefore blood temperature) fell rapidly after pentolinium, which would limit the maximum attainable tail skin temperature. Also, blood pressure (and therefore tail blood flow) would be greatly reduced by pentolinium. These two effects would combine to offset any increase in tail skin temperature evoked by ganglionic blockade. Moreover, it is known that in anaesthetized rats, abdominal sympathectomy produces an elevation in t_{18} to the same level as that observed during body heating (O'Leary *et al.*, 1985).

As to whether post-junctional α_2 -adrenoceptors provide the major mechanism of ongoing sympathetic vasoconstriction in the tail vasculature, propranolol (10 mg kg⁻¹, s.c.) produced only a small ($+2.9^\circ\text{C}$) increase in tail skin temperature accompanied by a 1°C fall in core temperature, arguing against a major role of β -adrenoceptors in the control of tail

blood flow in this species. Perhaps surprisingly, prazosin (0.3 mg kg^{-1} , s.c.) had no effect, indicating that α_1 -adrenoceptors do not play a major role in sympathetic vasomotor control within the tail. Early attempts to determine the relative contribution of post-junctional α_1 - vs. α_2 -adrenoceptors to vasoconstriction in the tail vasculature used proximal segments of the main ventral tail artery *in vitro*; whereas Itoh *et al.* (1983) clearly demonstrated an α_2 -adrenoceptor component, Medgett *et al.* (1984) were able to demonstrate an α_2 -adrenoceptor component only in tail arteries taken from hypertensive rats. The conclusions drawn by subsequent authors on the role of α_2 -adrenoceptors in the tail artery ranged from mere facilitation of the α_1 -adrenoceptor mediated response (Xiao & Rand, 1989) to a complete denial of their existence in this vessel (Marwood *et al.*, 1986). However, Medgett (1985) looked beyond the proximal tail artery and found that the contribution made by α_2 -adrenoceptors was greater in more distal segments of the vessel; our present data illustrate this in the intact, entire tail vascular bed, both *in vivo* and, under appropriate conditions, *in vitro*. It is also known that, in rings prepared from the rat tail artery, α_2 -adrenoceptor-mediated contractions are enhanced when the temperature is reduced from 37°C to 24°C (Harker *et al.*, 1991), which is close to the baseline tail skin temperatures in our experiments (where the ambient temperature was the standard housing temperature for laboratory rats).

The α_2 -adrenoceptor agonist, clonidine, itself produced a small increase in $t_{1/2}$ with a pronounced hypothermia; clearly this hypothermia was not due entirely to heat loss from the tail, as much larger increases in tail skin temperature were evoked by α_2 -adrenoceptor antagonists, associated with only modest decreases in core temperature. This suggests either cutaneous vasodilatation elsewhere or an inhibition of heat production as the cause of the clonidine hypothermia: a centrally mediated decrease in thermogenesis has previously been ascribed to this agent in the rat (Lin *et al.*, 1984). The small increase in $t_{1/2}$ may have been secondary to an initial increase in blood pressure. A similar hypothermia occurred after administration of the 5-HT_{1A} agonist, DP5-CT, without

any change in $t_{1/2}$, again serving to emphasize the specificity of the response to the α_2 -adrenoceptor antagonists. The finding that hydralazine did not increase $t_{1/2}$ suggests that α_2 -adrenoceptor antagonists do not cause generalized arterial vasodilatation within the tail. If their effect was mediated by opening arteriovenous anastomoses, which are known to be present in this vascular bed in the rat (Gammel & Hales, 1977), hydralazine might be expected to do the same, as it does open arteriovenous anastomoses, at least in the rabbit (Bolt & Saxena, 1984). Alternatively, it is possible that the tail arterioles are insensitive to hydralazine, although it is known that the proximal tail artery does relax to hydralazine *in vitro* (Ebeigbe & Aloamala, 1985). The most likely explanation is that the α_2 -adrenoceptor antagonists effect a redistribution of blood flow from the core of the tail to the surface either by an action on the arterial or venous side of the tail vasculature. It is known that such a redistribution of blood flow occurs in the rat tail *in vivo* as a local response to changes in the ambient temperature to which the tail is exposed (Raman *et al.*, 1983); perhaps the increased effectiveness of α_2 -adrenoceptor-mediated vasoconstriction as temperature is lowered (Harker *et al.*, 1991) contributes to this local thermal autoregulation.

In summary, blockade of α_2 -adrenoceptors within the rat tail *in vivo* elevates tail skin temperature, whereas activation of α_2 -adrenoceptors within the rat tail *in vitro* reduces tail skin temperature. We conclude that post-junctional α_2 -adrenoceptors in the vasculature of the rat tail play a major role in sympathetic vasoconstriction within this organ, controlling the flow and distribution of blood within the tail, and thereby thermoregulatory heat loss from its surface. This is analogous to their apparent role in the vascular beds of some human extremities, such as the fingertips (Coffman, 1991).

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References

- BOLT, G.R. & SAXENA, P.R. (1984). Interaction of atenolol with the systemic and regional hemodynamic effects of hydralazine in conscious renal hypertensive rabbits. *J. Pharmacol. Exp. Ther.*, **230**, 205–213.
- BROWN, C.M., MACKINNON, A.C., MCGRATH, J.C. SPEDDING, M. & KILPATRICK, A.T. (1990). α_2 -Adrenoceptor subtypes and imidazoline-like binding sites in the rat brain. *Br. J. Pharmacol.*, **99**, 803–809.
- BROWN, C.M., MACKINNON, A.C., REDFERN, W.S., HICKS, P.E., KILPATRICK, A.T., SMALL, C., RAMCHARAN, M., CLAGUE, R.U., CLARK, R.D., MACFARLAND, C.B. & SPEDDING, M. (1993). The pharmacology of RS-15385-197, a potent and selective α_2 -adrenoceptor antagonist. *Br. J. Pharmacol.*, **108**, 516–525.
- COFFMAN, J.D. (1991). Raynaud's phenomenon: an update. *Hypertension*, **17**, 593–602.
- COX, B. & LEE, T.F. (1977). Do central dopamine receptors have a physiological role in thermoregulation? *Br. J. Pharmacol.*, **61**, 83–86.
- EBEIGBE, A.B. & ALOAMAKA, C.P. (1985). Mechanism of hydralazine-induced relaxation of arterial smooth muscle. *Cardiovasc. Res.*, **19**, 400–405.
- GEMMELL, R.T. & HALES, J.R.S. (1977). Cutaneous arteriovenous anastomoses present in the tail but absent from the ear of the rat. *J. Anat.*, **124**, 355–358.
- GRANT, R.T. (1963). Vasodilatation and body warming in the rat. *J. Physiol.*, **167**, 311–317.
- HARKER, C.T., OUSLEY, P.J., BOWMAN, C.J. & PORTER, J.M. (1991). Cooling augments α_2 -adrenoceptor-mediated contractions in rat tail artery. *Am. J. Physiol.*, **260**, H1166–H1171.
- ITOH, T., KITAMURA, K. & KURIYAMA, H. (1983). Roles of extra-junctional receptors in the response of guinea-pig mesenteric and rat tail arteries to adrenergic nerves. *J. Physiol.*, **345**, 409–422.
- LIN, M.T., SHIAN, L.R. & LEU, S.Y. (1984). Clonidine-induced hypothermia: possible involvement of cholinergic and serotonergic mechanisms. *Naunyn-Schmied. Arch. Pharmacol.*, **326**, 124–128.
- LITTLE, R.A. & STONER, H.B. (1968). The measurement of heat loss from the rat's tail. *Q. J. Exp. Physiol.*, **53**, 76–83.
- MACLEAN, M.R. & MCGRATH, J.C. (1990). Effects of pre-contraction with endothelin-1 on α_2 -adrenoceptor- and (endothelium-dependent) neuropeptide Y-mediated contractions in the isolated vascular bed of the rat tail. *Br. J. Pharmacol.*, **101**, 205–211.
- MARWOOD, J.F., CHAPMAN, K.L. & STOKES, G.S. (1986). Studies that question the existence of α_2 -adrenoceptors in tail arteries of normotensive Sprague-Dawley rats. *J. Pharmacol. Exp. Ther.*, **238**, 267–272.
- MEDGETT, I.C. (1985). α_2 -Adrenoceptors mediate sympathetic vasoconstriction in distal segments of rat tail artery. *Eur. J. Pharmacol.*, **108**, 281–287.
- MEDGETT, I.C., HICKS, P.E. & LANGER, S.Z. (1984). Smooth muscle α_2 -adrenoceptors mediate vasoconstrictor responses to exogenous norepinephrine and to sympathetic stimulation to a greater extent in spontaneously hypertensive than in Wistar Kyoto rat tail arteries. *J. Pharmacol. Exp. Ther.*, **231**, 159–165.
- O'LEARY, D.S., JOHNSON, J.M. & TAYLOR, W.F. (1985). Mode of neural control mediating rat tail vasodilatation during heating. *J. Appl. Physiol.*, **59**, 1533–1538.
- PAXINOS, G. & WATSON, C. (1982). *The Rat Brain in Stereotaxic Coordinates*. Sydney: Academic Press.
- RAYMAN, E.R., ROBERTS, M.F. & VANHUUSE, V.J. (1983). Body temperature control of rat tail blood flow. *Am. J. Physiol.*, **245**, R426–R432.

- RAND, R.P., BURTON, A.C. & ING, T. (1965). The tail of the rat, in temperature regulation and acclimatization. *Can. J. Physiol. Pharmacol.*, **43**, 257–267.
- REDFERN, W.S. & CLAGUE, R.U. (1991). Effects of RS-15385-197 and other α_2 -adrenoceptor antagonists on tail skin temperature in unanaesthetized rats. *Br. J. Pharmacol.*, **104**, 50P.
- REDFERN, W.S., MACLEAN, M.R. & MCGRATH, J.C. (1994). α -Adrenoceptor antagonists elevate tail skin temperature in the rat by an action within the tail, not the brain. *Br. J. Pharmacol.*, **113**, 101P.
- REDFERN, W.S., MACKINNON, A.C., BROWN, C.M., MARTIN, A.B., KILPATRICK, A.T., CLAGUE, R.U. & SPEDDING, M. (1993). Modulation of central noradrenergic function by RS-15385-197. *Br. J. Pharmacol.*, **108**, 526–533.
- TEMPLETON, A.G.B., MACMILLAN, J., MCGRATH, J.C., STOREY, N.D. & WILSON, V.G. (1989). Evidence for prazosin-resistant, rauwolscine-sensitive α -adrenoceptors mediating contractions in the isolated vascular bed of the rat tail. *Br. J. Pharmacol.*, **97**, 563–571.
- THOMSON, G.E. & STEVENSON, J.A.F. (1965). The temperature response of the male rat to treadmill exercise, and the effect of anterior hypothalamic lesions. *Can. J. Physiol. Pharmacol.*, **43**, 279–287.
- THORINGTON, R.W. (1966). *The Biology of Rodent Tails: A Study of Form and Function*. Report of Project 823, Document Number AAL-TR-65-8 from Arctic Aeromedical Laboratory, Fort Wainwright, Alaska.
- WILSON, K.M. & FREGLY, M.J. (1985). Factors affecting angiotensin II-induced hypothermia in rats. *Peptides*, **6**, 695–701.
- YOUNG, A.A. & DAWSON, N.J. (1982). Evidence for on-off control of heat dissipation from the tail of the rat. *Can. J. Physiol. Pharmacol.*, **60**, 392–398.
- XIAO, X.-H. & RAND, M.J. (1989). α_2 -Adrenoceptor agonists enhance responses to certain other vasoconstrictor agonists in the rat tail artery. *Br. J. Pharmacol.*, **96**, 539–546.

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