J. exp. Biol. 152, 425–439 (1990)
Printed in Great Britain © The Company of Biologists Limited 1990

AUTONOMIC REGULATION OF CUTANEOUS VASCULAR RESISTANCE IN THE BULLFROG *RANA CATESBEIANA*

BY GARY M. MALVIN AND CINDY RIEDEL

Research Division, Lovelace Medical Foundation, 2425 Ridgecrest Dr SE, Albuquerque, NM 87108, USA

Accepted 19 April 1990

Summary

To gain a better understanding of the regulation of cutaneous blood flow in the bullfrog, the vascular innervation, vasoactivity and adrenoceptor types of the cutaneous vasculature were investigated using a pump-perfused skin preparation. Stimulation of cranial nerve I, the vagal ganglion, sympathetic ganglion 1 and sometimes sympathetic ganglion 2 caused cutaneous vascular resistance (CVR) to increase. Stimulation of cranial nerve IX and spinal nerves 1 and 2 had no effect on CVR. The response to stimulation of sympathetic ganglion 1 was antagonized by phentolamine but not by atropine. Phentolamine, atropine and α,β-methylene ATP had no effect on the response to vagal stimulation. Both epinephrine (EPI) and norepinephrine (NE) increased CVR, with EPI being more potent than NE. The minimum concentrations of EPI and NE required for a significant change in CVR were much higher than plasma catecholamine levels reported for resting bullfrogs. Phentolamine antagonized, but propranolol had no effect on, the responses to the catecholamines. Isoproterenol caused small decreases in CVR which were abolished by propranolol. Acetylcholine was a weak vasodilator. The results indicate that the cutaneous vasculature has two types of vasomotor nerves: sympathetic nerves that are probably adrenergic, and other nerves that are non-adrenergic/non-cholinergic and which do not use ATP as a transmitter. Although catecholamines are vasoactive, the sensitivity of the cutaneous vasculature to EPI and NE is probably too low to allow a direct regulatory role of these hormones on CVR. There is no evidence for cholinergic regulation of CVR. Both α- and β-adrenoceptors are present in the cutaneous vasculature. α-Adrenoceptors mediate the constrictor responses to sympathetic nerve stimulation and catecholamine administration. It is unlikely that β-adrenoceptors play a significant role in regulating CVR.

Introduction

In amphibians, skin is a major respiratory and osmoregulatory organ. The exchange of gases across the skin (Malvin and Hlastala, 1989), as well as ions and water (Christensen, 1974; Mahany and Parsons, 1978), is regulated in part by

Key words: sympathetic nerves, adrenergic nerves, non-adrenergic/non-cholinergic nerves, Amphibia, adrenoceptors, acetylcholine, cutaneous blood flow, skin.

cutaneous blood flow. The mechanisms controlling cutaneous blood flow, however, are poorly understood. Several studies have shown that cutaneous blood flow can be regulated by sympathetic innervation of the cutaneous vasculature (Langley, 1911; Krogh *et al.* 1922; Smith, 1976). However, little is known of the neural pathways, transmitters and receptors involved in such regulation, nor has possible hormonal regulation of the cutaneous vasculature by circulating catecholamines been evaluated.

The purpose of this study was to characterize further the vascular innervation, the adrenoceptors and the vascular sensitivity to catecholamines of bullfrog skin. There were four specific goals: (1) to determine whether nerves associated with the vagal ganglion, the first two cervical sympathetic ganglia, cranial nerves IX and X and spinal nerves 1 and 2, can regulate cutaneous vascular resistance (CVR); (2) to determine whether catecholamines, acetylcholine or ATP are among the transmitters used by cutaneous vasomotor nerves; (3) to determine the dose–response relationships of epinephrine (EPI) and norepinephrine (NE) in the cutaneous vasculature and to assess the possibility of hormonal control of CVR by these substances; and (4) to determine the adrenoceptor types in the cutaneous vasculature. All experiments were performed on a pump-perfused skin preparation in which the cutaneous artery was perfused at constant flow while perfusion pressure was measured.

Materials and methods

Bullfrogs, *Rana catesbeiana*, were obtained from an animal dealer and housed in a large aquarium at 24°C at least 1 week before experimentation. Body mass ranged from 100 to 691 g (mean=308 g; S.D.=115 g; N=68).

Pump-perfused skin preparation

A bullfrog was double-pithed and placed supine on an elevated wire mesh. A midline incision through the pectoral girdle was made to expose the aortic arches and large veins entering the heart. Heparin (50 units; Elkins-Sinn, Inc.) was injected into the ventricle to prevent clotting in the preparation. A cannula (PE 50) was placed in the left anterior vena cava which receives venous effluent from the portions of skin to be perfused. Since venous pressure in frogs is approximately 0 kPa (Jones and Shelton, 1972), the distal end of the cannula was positioned 2 cm below the level of the heart. This ensured that outflow pressure in the vein was approximately 0 kPa to compensate for the cannula resistance that caused an approximately 0.27 kPa fall in pressure along its length at the flow rates used. The left pulmonary artery just proximal to the lung was then ligated. A cannula was placed in the left pulmocutaneous artery so that the skin supplied by the left cutaneous artery could be selectively perfused at constant flow. A roller pump (Masterflex 7523-10) withdrew perfusate from a reservoir and pumped it at constant flow through the cannula in the pulmocutaneous artery. The perfusate was bubbled with a gas mixture of 98 % O_2 and 2 % CO_2 delivered by a Wösthoff

gas-mixing pump. Perfusion pressure was measured with a pressure transducer (Statham) connected to the perfusion line *via* a T-connection. Just distal to the T-connection was a thick rubber injection port through which small volumes of drugs could be administered (Fig. 1). Flow through the arterial cannula was adjusted so that perfusion pressure was approximately 2 kPa. Mean arterial pressure in bullfrogs at rest is 1.96 kPa (Herman *et al.* 1986). Flow was determined after the experiments by cutting the pulmocutaneous artery at the cannula tip and then measuring the volume of perfusate pumped over 5 min. Perfusion pressure had no effect on pump flow. The perfusate was a modified Ringer's solution containing (in $mmol\,l^{-1}$): NaCl, 76.1; $NaHCO_3$, 30.7; NaH_2PO_4, 1.4; KCl, 2.5; $MgSO_4$, 3.1; $CaCl_2$, 4.0; glucose, 5.6; and $30\,g\,l^{-1}$ dextran (M_r 80 000). All experiments were made at room temperature (22–24 °C).

The tissue perfused in this preparation was the skin on the sides and back. This was determined by adding Methylene Blue to the perfusate in four preparations and noting the areas of skin that were dyed after 20 min. Dye was never observed in areas other than the sides and back.

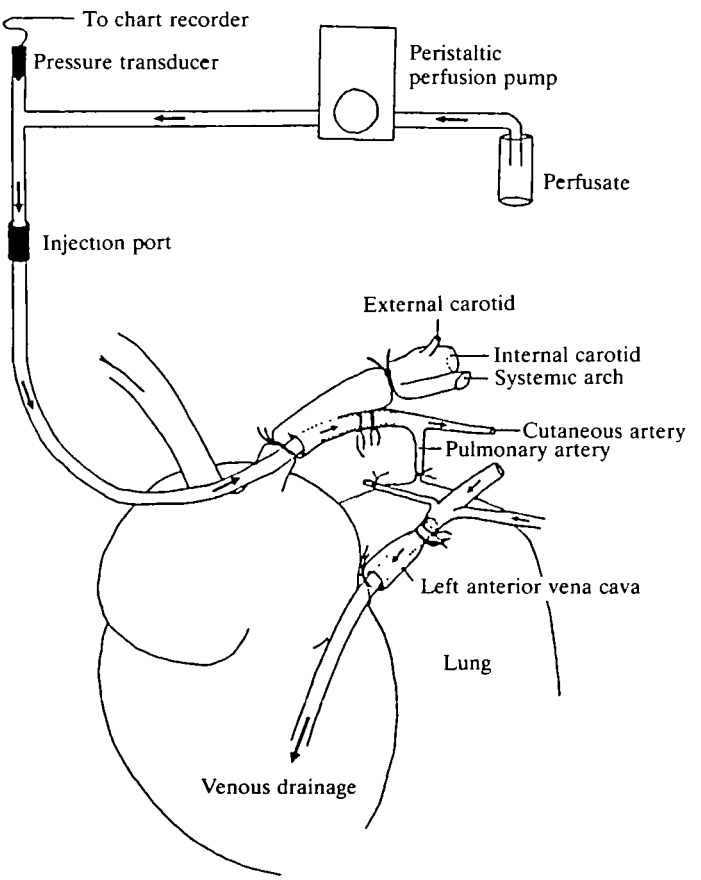

Fig. 1. Diagram of the experimental apparatus.

Nerve stimulation experiments

The names of the nerves follow the nomenclature of Pick (1970; Fig. 2 of this paper). The vagal ganglion, sympathetic ganglia 1 and 2, cranial nerves IX (glossopharyngeal) and X (vagus) and the first two spinal nerves on the left side of the animal were exposed by an incision in the roof of the mouth. The cutaneous artery and vein were cannulated as described and perfusion of the skin begun. 20 min after the initiation of skin perfusion, the nerves were electrically stimulated one at a time. The order of nerve stimulation was randomized. Stimulation was performed with bipolar platinum electrodes placed beneath the nerves or ganglia. 5 mA pulses of 1 ms duration at 5 Hz were delivered in a square-wave pattern for 20 s by a Grass S88 stimulator with a Grass stimulus isolation unit (PSIU6). Preliminary experiments determined that these parameters were well above threshold but below the stimulus required for a maximal response in the sympathetic ganglia and cranial nerve X. If a response was obtained, perfusion

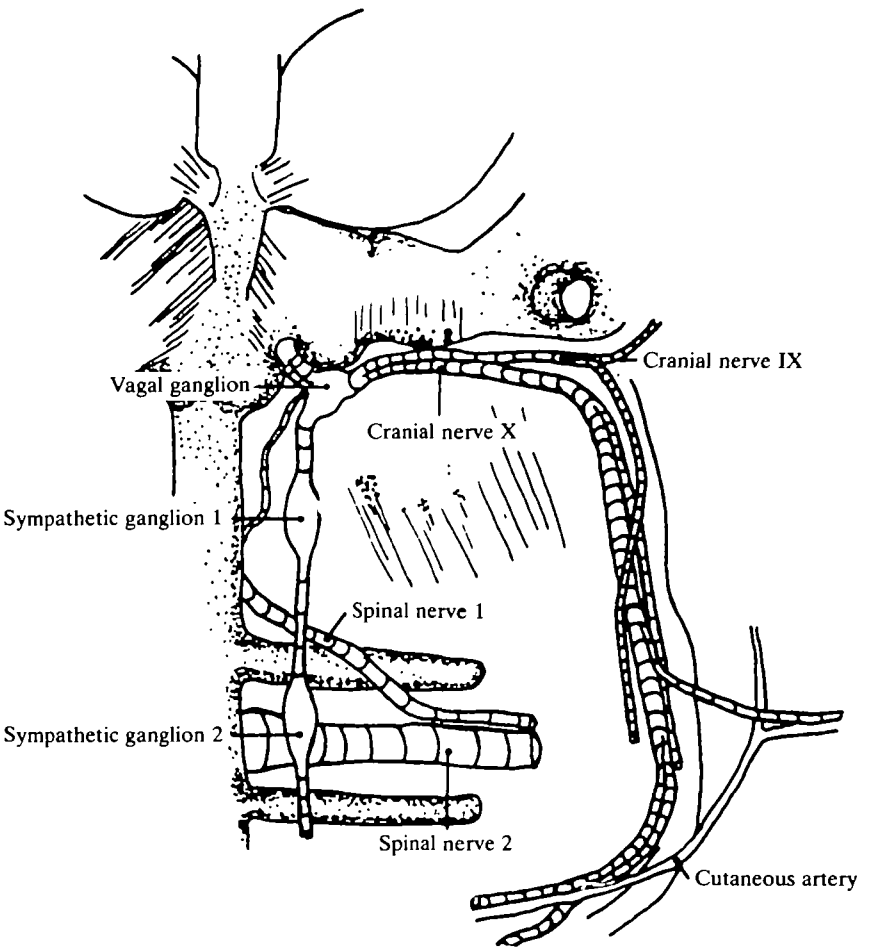

Fig. 2. Diagram of the nerves and ganglia stimulated; ventral view.

pressure was allowed to return to prestimulation levels (approximately 10–20 min) before the next nerve was stimulated.

To determine neural pathways, nerves were stimulated again before and after neural connections had been selectively severed. Specifically, sympathetic ganglion 2 was stimulated, then the connections between the first and second sympathetic ganglia were severed and sympathetic ganglion 2 was stimulated again. Sympathetic ganglion 1 was then stimulated, the connections between the vagal ganglion and the first sympathetic ganglion were cut and the first sympathetic ganglion was then stimulated. Next, the vagal ganglion was stimulated, then cranial nerves IX and X were cut just lateral to the vagal ganglion and then the vagal ganglion was stimulated. Finally, cranial nerve X was stimulated.

To determine whether the nerves innervating the cutaneous vasculature were adrenergic, cholinergic or neither, sympathetic ganglion 1 and cranial nerve X were stimulated before and after adrenergic and cholinergic antagonists had been added to the perfusate. In six frogs, sympathetic ganglion 1 and cranial nerve X were stimulated as previously described with 20 min between the two stimulations. Then the α-adrenoceptor antagonist phentolamine (10^{-5} mol l^{-1}; Regitine, CIBA Pharmaceutical Co.) was added to the perfusate. 30 min later the two nerves were stimulated as before. Atropine (10^{-5} mol l^{-1}; Sigma Chemical Co.), a muscarinic antagonist, was then added to the perfusate and 30 min later the same two nerves were stimulated. This protocol was repeated on six additional frogs but atropine was added before phentolamine.

Another four frogs were tested to determine if cutaneous vasomotor nerves in the vagus use ATP as a transmitter. Animals were prepared as described above. Cranial nerve X was stimulated, then α,β-methylene ATP (10^{-4} mol l^{-1}; Sigma Chemical Co.) was added to the perfusate. [α,β-methylene ATP is a methylated analogue of ATP which desensitizes P_2-purinoceptors (Kasakov and Burnstock, 1983). P_2-purinoceptors mediate ATP-induced responses in many different types of blood vessels (Burnstock and Brown, 1981; Su, 1981).] 30 min later, the vagus was stimulated again. The vagus was stimulated a third time 30 min after 10^{-3} mol l^{-1} α,β-methylene ATP had been added to the perfusate.

Dose–response relationships for EPI and NE

The cutaneous vasculature was perfused as described. 20 min after perfusion had been initiated, either EPI (Adrenalin, Parke-Davis; $N=7$) or NE (Levophed, Winthrop-Breon; $N=8$) was added cumulatively to the perfusate. Dose–response relationships between 1.8×10^{-8} and 1.8×10^{-4} mol l^{-1} were produced. Perfusate catecholamine concentration was increased by 0.5 log units after CVR became stable (approximately 10–20 min).

Adrenoceptor types and the response to acetylcholine

Two groups of experiments evaluated the adrenoceptor types in the cutaneous vasculature. In one group, the response of the cutaneous vasculature to EPI and NE was tested before and after α- and β-adrenoceptor antagonists (phentolamine

and propranolol, respectively) had been added to the preparation. The other group of experiments tested the effect of selective α- and β-adrenoceptor agonists (phenylephrine and isoproterenol, respectively). This second group also evaluated the effects of acetylcholine on the cutaneous vasculature.

In the first set of experiments, two doses (0.1 and 1.0 nmol) of both EPI and NE were administered in a random order through the injection port by a Hamilton syringe. Injection volume was always 2 μl, which had only a small, brief effect on perfusion pressure. Injections usually caused a transient increase in CVR. Succeeding injections were administered only after CVR had returned to the basal level (approximately 5–20 min). After the fourth injection, either propranolol (10^{-6} mol l^{-1}; Inderal, Ayerst Laboratories, Inc.; N=7) or phentolamine (10^{-6} mol l^{-1}; N=8) was added to the perfusate. 30 min later, the four different catecholamine injections were administered in a random order. Controls (N=8) were performed over the same period by repeating the above protocol without adding an adrenoceptor antagonist.

In the second set of experiments (N=10), phenylephrine (10.0 and 100 nmol; Neosynephrine, Winthrop Laboratories, Inc.), isoproterenol (0.1, 1.0 and 10.0 nmol; Sigma Chemical Co.) and acetylcholine (0.1, 1.0 and 10.0 nmol; Sigma Chemical Co.) were injected into the perfusion line in random order. Drugs were injected only after CVR had returned to the basal level after the preceding injection (approximately 5–20 min). Then KCl was added to the perfusate to produce a KCl concentration of 40 mmol l^{-1}. The added KCl elevated CVR. This was done to determine if the small decreases in CVR caused by isoproterenol and acetylcholine could be augmented by first increasing CVR. Then the same doses of isoproterenol and acetylcholine were administered as before. Finally, propranolol (10^{-6} mol l^{-1}) was added to the perfusate and 30 min later the three doses of isoproterenol were given to determine if the responses to isoproterenol were mediated by β-adrenoceptors.

Data analysis

Cutaneous vascular resistance was determined by dividing perfusion pressure by perfusate flow and then subtracting the resistance of the arterial cannula. Cannula resistance was determined after the experiment by cutting the pulmocutaneous artery at the cannula tip and dividing the resulting perfusion pressure by the flow.

Responses to nerve stimulation and drug injections into the perfusion line were expressed as the maximal percentage change in CVR recorded after the stimulus. Cumulative dose–response curves to EPI and NE were analyzed for (1) the minimum concentration required for a significant increase in CVR, (2) the maximal CVR caused by the catecholamines and (3) the median effective doses (ED_{50}), calculated by expressing CVR as a percentage of the maximal response, performing a logit–log transformation on the data and then fitting a line to the data with a least-squares linear regression.

Paired t-tests were used to evaluate: (1) differences in responsiveness to catecholamine injections caused by adrenoceptor antagonists and by time; (2) the

effects of adrenoceptor agonists and acetylcholine; and (3) the difference in basal CVR at the beginning and at the end of the experiment. The maximal response to, and ED_{50} values of, EPI and NE were compared with an unpaired *t*-test. One-way ANOVA, followed if necessary by a Newman–Keuls test, evaluated differences in responsiveness to (1) nerve stimulation caused by atropine and/or phentolamine and (2) the two doses of α-,β-methylene ATP. Statistical significance was set at a level of $P=0.05$.

Results

At the beginning of the experiments, mean (±S.D.) perfusion pressure was 2.3±0.6 kPa and CVR was 3.1±1.5 kPa min kg body mass ml^{-1}. In experiments without sustained increases in CVR due to the addition of KCl or catecholamines to the reservoir of perfusate there was no change in basal CVR between the beginning and the end of the experiment ($P>0.5$).

Nerve stimulation experiments

Changes in CVR produced by nerve stimulation are shown in Figs 3 and 4. Stimulation of the vagal ganglion, sympathetic ganglion 1 and cranial nerve X always produced increases in CVR. Sympathetic ganglion 2 stimulation increased CVR in two frogs but had no effect in the other two. Stimulation of cranial nerve IX and spinal nerves 1 and 2 had no effect on CVR in the four frogs tested. The elevation of CVR in response to nerve stimulation lasted several minutes. There was no apparent difference in the time course of the response between vagal and sympathetic ganglia stimulation. Stimulating sympathetic ganglion 2 after cutting between the first and second sympathetic ganglia never altered CVR. This sectioning had no effect on changes in CVR caused by stimulation of sympathetic ganglion 1. Cutting between sympathetic ganglion 1 and the vagal ganglion eliminated the response to stimulation of sympathetic ganglion 1, but had no effect on the response to stimulation of the vagal ganglion. Sectioning cranial nerve X lateral to the vagal ganglion eliminated the response to stimulation of the vagal ganglion but had no effect on the response to stimulation of cranial nerve X.

Addition of phentolamine to the perfusate decreased the response to stimulation of sympathetic ganglion 1 by 78% ($P<0.01$), but had no significant effect on the response to stimulation of cranial nerve X ($P>0.07$; Fig. 5). Subsequent addition of atropine to the perfusate (already containing phentolamine) did not change the response to stimulation of either of these nerves ($P>0.1$; Fig. 5). Adding atropine to the perfusate before phentolamine had no effect on the response to nerve stimulation ($P>0.1$; Fig. 5). Adding phentolamine after atropine caused a 68% decrease in the response to stimulation of sympathetic ganglion 1 ($P<0.01$), but did not alter the response to stimulation of the cranial nerve X ($P>0.1$; Fig. 5). Neither 10^{-4} nor 10^{-3} mol l^{-1} α-,β-methylene ATP affected the response to stimulation of sympathetic ganglion 1 and cranial nerve X

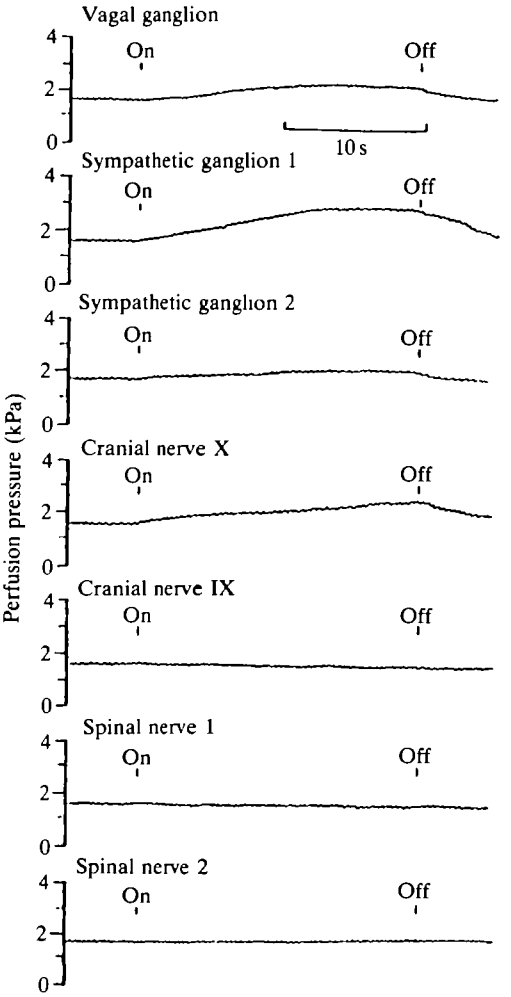

Fig. 3. Tracings from an experiment showing the response to selective nerve stimulation. Each nerve was stimulated with 5 mA pulses of 1 ms duration at 5 Hz for 20 s. The increase in perfusion pressure indicates an increase in cutaneous vascular resistance.

($P>0.1$). Atropine, phentolamine and α-,β-methylene ATP had no effect on basal CVR ($P>0.1$).

Dose–response relationships for epinephrine and norepinephrine

Addition of EPI and NE to the perfusate caused dose-dependent increases in CVR up to a concentration of approximately 10^{-5} mol l^{-1} (Fig. 6). Higher catecholamine concentrations caused CVR to fall. The lowest concentration of EPI tested which significantly raised CVR was 1.8×10^{-7} mol l^{-1} ($P=0.048$), while that for NE was 1.8×10^{-6} mol l^{-1} ($P=0.003$). There was no significant difference in the maximal CVR caused by EPI and NE ($P>0.5$). The ED_{50} for EPI at

$2.1\pm0.7\times10^{-6}$ mol l^{-1} was significantly different ($P=0.035$) from the ED_{50} for NE at $4.8\pm0.9\times10^{-6}$ mol l^{-1}.

Adrenoceptor types and the response to acetylcholine

Injections of EPI and NE into the perfusion line caused transient increases in

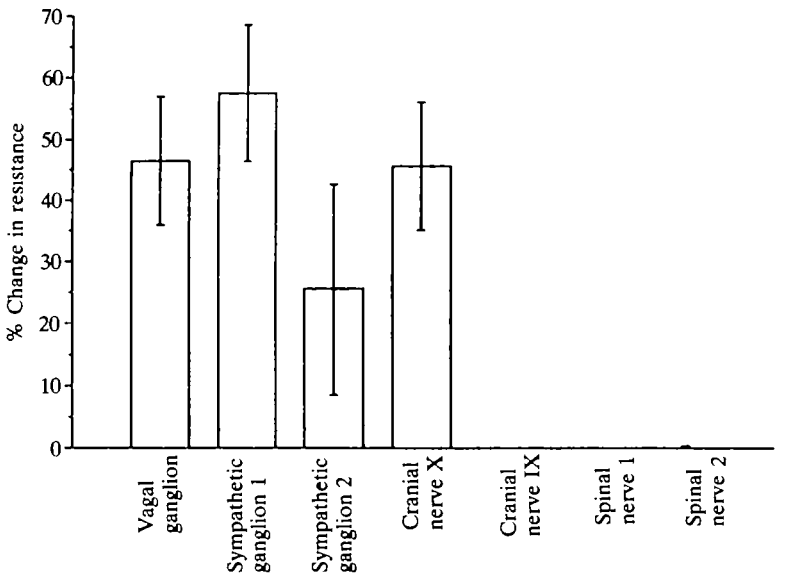

Fig. 4. Effects of selective nerve stimulation on cutaneous vascular resistance. Stimulation of spinal nerves 1 and 2 and of cranial nerve IX had no effect on cutaneous vascular resistance. Values are means±S.E.M. ($N=4$).

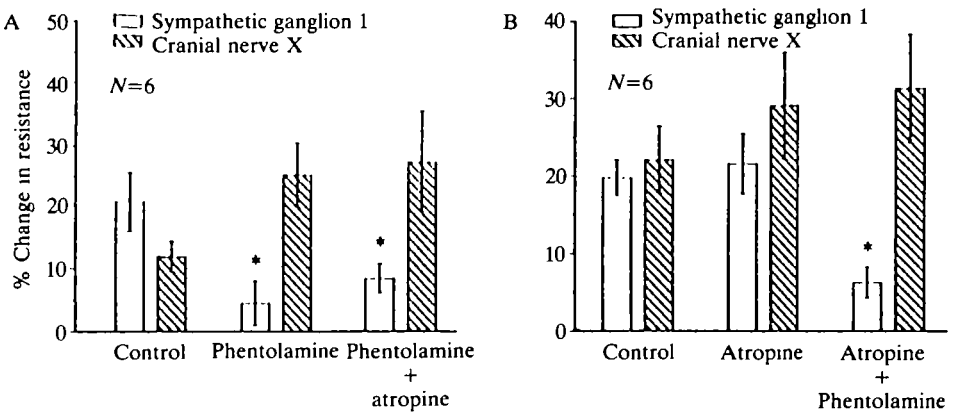

Fig. 5. (A) Effects of phentolamine alone and phentolamine plus atropine on responses to stimulation of cranial nerve X and sympathetic ganglion 1. (B) Effects of atropine alone and atropine plus phentolamine on responses to stimulation of cranial nerve X and sympathetic ganglion 1. Values are means±S.E.M. ($N=6$). Asterisks indicate that the response in the presence of the antagonist(s) was significantly less than the response in the absense of antagonist(s) ($P<0.05$).

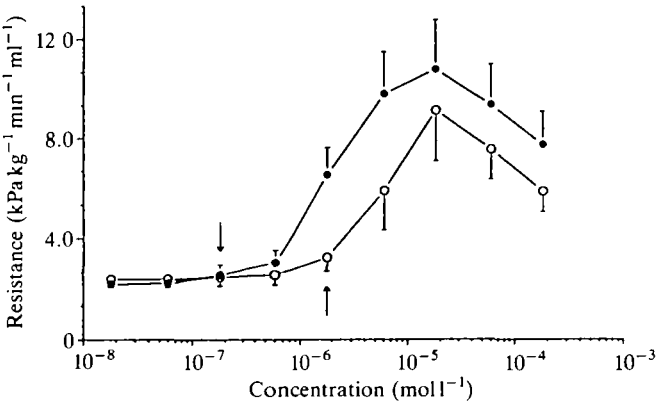

Fig. 6. Dose–response relationship of the cutaneous vasculature to epinephrine (●) (N=7) and norepinephrine (○) (N=8). Values are means+S.E.M. Arrows indicate the lowest concentration tested that caused a significant increase in cutaneous vascular resistance (P<0.05).

CVR lasting for approximately 5–20 min. In the control experiments, the magnitude of the responses to repeat injections after 30 min was not different from that of the initial injections (P>0.29; Fig. 7). Phentolamine reduced the responses to NE and EPI (P<0.05), except for the response to 0.1 nmol NE (P>0.21; Fig. 7). Propranolol had no effect on the responses (P>0.1; Fig. 7). Neither antagonist had an effect on basal CVR (P>0.4).

1 and 10 nmol of phenylephrine caused significant increases in CVR of 11.2±4.3 and 28.1±7.0 %, respectively (P<0.034). Without KCl (40 mmol l^{-1}) added to the perfusate to raise CVR, isoproterenol decreased CVR at the three doses tested (P<0.03, Fig. 8). However, the mean changes were always less than 9 %. Addition of KCl to the perfusate raised CVR by 76.9±20.2 %. Administration of isoproterenol under these conditions of elevated basal CVR also decreased CVR, but the percentage decrease in CVR was not greater than before KCl was added (Fig. 8). Propranolol abolished the changes in CVR caused by isoproterenol (P>0.4; Fig. 8). Without extra KCl added to the perfusate, acetylcholine decreased CVR by 7 % (P<0.002) at the lowest dose tested (0.1 nmol; Fig. 8). The two higher doses (1 and 10 nmol) of acetylcholine had no effect on CVR (P>0.09). With CVR elevated by the addition of KCl to the perfusate, acetylcholine caused significant decreases in CVR at the two lowest doses (P<0.004; Fig. 8). The magnitudes of the decreases were under 13 %. The highest dose of acetylcholine had no effect on CVR (P=0.08).

Discussion

In the intact bullfrog, most blood flowing through the cutaneous artery goes to the skin on the sides and back, although some of this blood reaches other skin

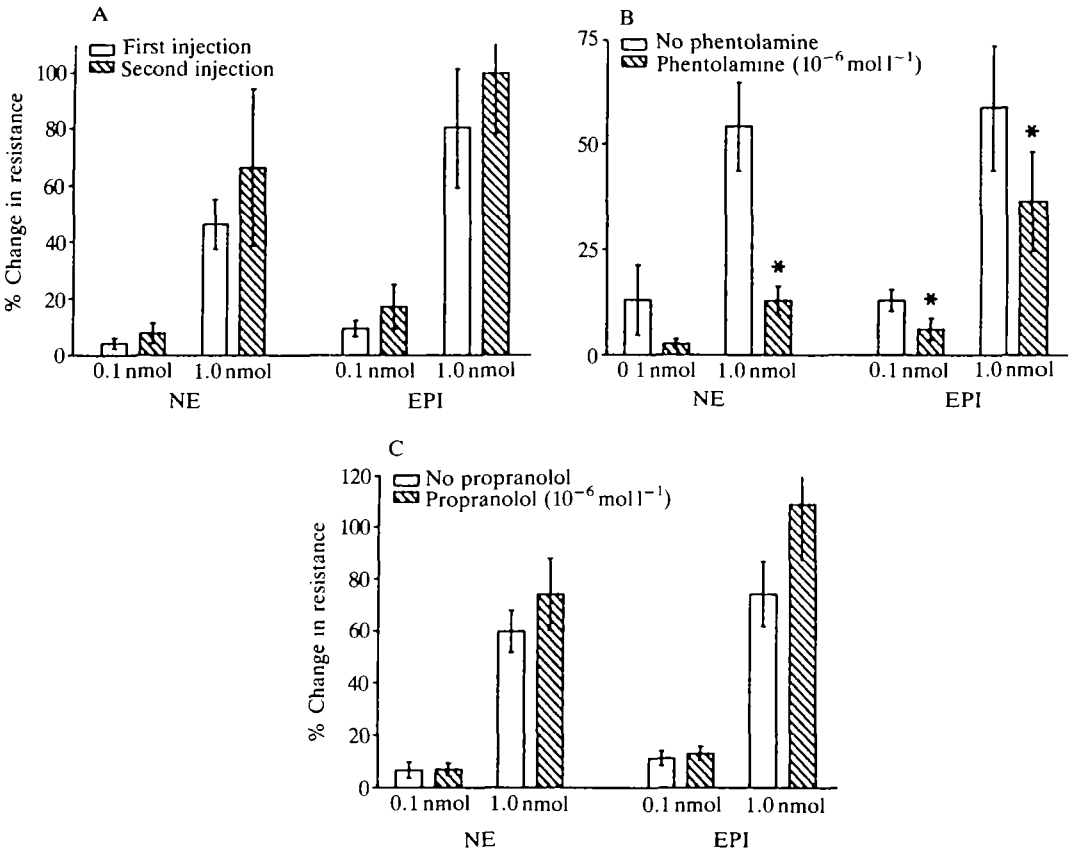

Fig. 7. (A) Effect of time on the responses of the cutaneous vasculature to injections of epinephrine and norepinephrine. 30 min separated the two sets of injections. There were no significant differences between the effects of the first and second injections ($P>0.05$; $N=8$). (B) Effect of phentolamine on the responses of the cutaneous vasculature to injections of EPI and NE. Asterisks indicate a significant difference between the responses before and after the addition of phentolamine ($P<0.05$; $N=8$). (C) Effect of propranolol on the responses of the cutaneous vasculature to injections of EPI and NE. There were no significant differences between the responses before and after the addition of propranolol ($P>0.05$; $N=7$). Values are means±S.E.M.

areas (Moalli *et al.* 1980). In the preparation of this study, only the skin of the sides and back was perfused. It is not known why the cutaneous artery supplies other areas *in vivo* but not in a pump-perfused preparation.

The preparation remained viable throughout the experimental periods. There was no decrease in responsiveness to EPI and NE injections in the control experiments. Responsiveness to vagal stimulation did not decrease over time and responsiveness to stimulation of sympathetic ganglion 1 decreased only after the addition of phentolamine to the perfusate. In addition, there was no change in basal CVR during the experiments.

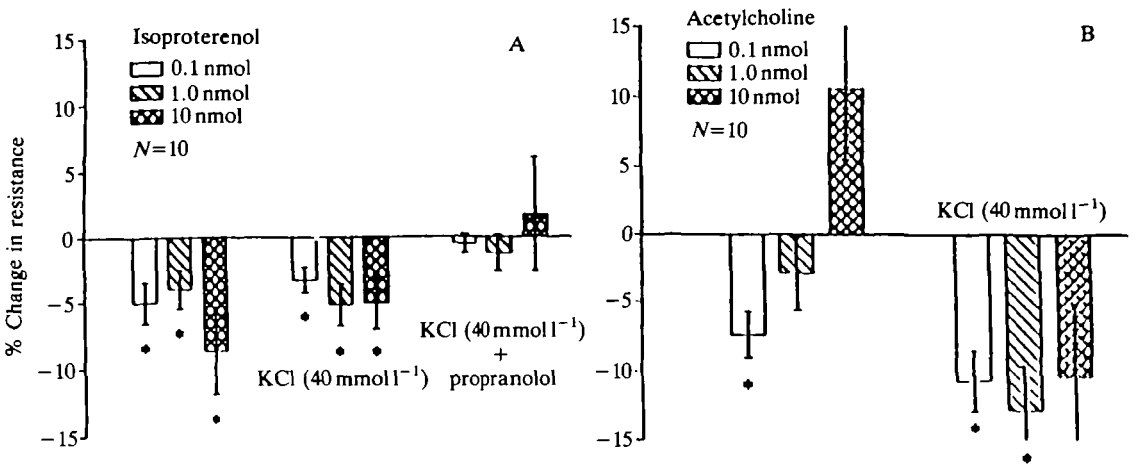

Fig. 8. (A) Responses of the cutaneous vasculature to isoproterenol with and without 40 mmol l^{-1} KCl and propranolol added to the perfusate. The added KCl increased basal cutaneous vascular resistance (CVR) by 76.9±20.2 %. Asterisks indicate that the isoproterenol injection caused a significant change in CVR ($P<0.05$). (B) Responses of the cutaneous vasculature to acetylcholine with and without 40 mmol l^{-1} KCl added to the perfusate. Asterisks indicate that the acetylcholine injection caused a significant change in CVR ($P<0.05$). Values are means±S.E.M. ($N=10$).

It is not known how the basal contractile state of the cutaneous vasculature in this preparation compares to that in the intact animal. However, there was some myogenic tone in the vasculature because isoproterenol and acetylcholine produced decreases in CVR. The basal resistance of the perfused preparation was probably greater than the resistance of the skin vasculature perfused by the cutaneous artery *in vivo*. Moalli *et al.* (1980) reported cutaneous arterial flows of 2.6 ml min^{-1} kg^{-1} during air-breathing and 1.3 ml min^{-1} kg^{-1} during diving in intact animals. In this study, mean basal perfusion flow was 0.74 ml min^{-1} kg^{-1}. The reason for this difference may, in part, be related to the smaller skin region supplied by the cutaneous artery in this preparation than *in vivo*.

The magnitude of the CVR increases observed with this preparation may have been less than would have been obtained if a constant-pressure, variable-flow setup had been used. In this preparation, for example, an increase in CVR causes perfusion pressure to increase, and such an increase may partially counteract the increase in CVR by acting to distend vessels. The degree of this possible effect of changing perfusion pressure on CVR is not known. However, a variable-pressure preparation may approximate some physiological situations; for example, lung ventilation in amphibians causes blood pressure to increase (Segura *et al.* 1981) and cutaneous blood flow to decrease (Moalli *et al.* 1980; Malvin and Hlastala, 1986*b*).

This study showed that the bullfrog cutaneous circulation receives sympathetic innervation because stimulation of sympathetic ganglia 1 increased cutaneous

vascular resistance. In half of the animals tested, stimulation of sympathetic ganglion 2 produced a response indicating that in some animals cutaneous sympathetic nerves are associated with that ganglion as well. It was shown that these nerves travel anteriorly in the sympathetic chain to join the vagus, because cutting the chain posterior to the stimulation site had no effect on the response to stimulation, but cutting anterior to the stimulation site abolished the response. It is likely that these nerves release EPI, which stimulates α-adrenoceptors. Epinephrine is the most common catecholamine released by adrenergic nerves in amphibians (Burnstock, 1969) and most of the response to stimulation of sympathetic ganglion 1 was blocked by phentolamine. It is not known whether the remaining response after blockade was due to incomplete blockage of α-adrenoceptors or to other transmitters.

Sympathetic innervation of the cutaneous circulation exists in other amphibians as well. Early studies on *Rana temporaria* showed that stimulation of the posterior sympathetic ganglia produced vasoconstriction in the skin of the foot web (Langley, 1911; Krogh *et al.* 1922). Using a preparation similar to the one of this study, Smith (1976) stimulated the cervical sympathetic connective in the toad *Bufo marinus* and increased vascular resistance in the skin supplied by the cutaneous artery. In addition, he demonstrated histologically the presence of adrenergic varicosities in the proximal segment of the cutaneous artery.

The lack of an effect of phentolamine on the response to vagal stimulation indicates that non-adrenergic nerves also travel in the vagus and innervate the cutaneous vasculature. These nerves are probably not cholinergic because atropine had no effect on the response to vagal stimulation. In addition, they do not appear to use ATP as a transmitter, since α,β-methylene ATP had no effect on vagal stimulation. It is possible that the transmitter is a peptide, but that possibility was not evaluated.

Both EPI and NE are pressor agents in the cutaneous vasculature, suggesting that circulating catecholamines may directly influence CVR. However, plasma [EPI] and [NE] in resting bullfrogs are 2.1×10^{-9} mol l^{-1} and 4.8×10^{-10} mol l^{-1}, respectively (Wilson *et al.* 1988), several orders of magnitude less than the minimum concentrations required for a significant increase in CVR in this present study. Consequently, if the sensitivity of the cutaneous vasculature to catecholamines is the same *in vivo* as that measured in this study, then these hormones do not directly influence CVR in the resting animal. Various stresses increase plasma [EPI] and [NE], so it is possible that under some circumstances plasma [EPI] and [NE] could rise enough to affect CVR. Unfortunately, the amount that circulating catecholamines levels can rise in the bullfrog is not known. In other amphibian species, the increase in plasma catecholamine levels in response to exercise and handling is less than 10-fold (Wahlqvist and Cambell, 1988; Bourgeois *et al.* 1978). Thus, a direct role for circulating catecholamines in the control of CVR is doubtful.

The cutaneous vasculature appears to contain both α- and β-adrenoceptors. The presence of α-adrenoceptors is supported by phentolamine antagonizing the

responses to sympathetic nerve stimulation and to EPI and NE injection, and by the increase in CVR induced by phenylephrine. β-Adrenoceptors may also be present because isoproterenol produced decreases in CVR which were antagonized by propranolol. However, it is unlikely that β-receptors play a significant role in regulating CVR. Decreases in CVR caused by isoproterenol were small (<10%) and propranolol had no effect on the responses to EPI and NE. α-Adrenoceptors are also probably present in the foot web. Burggren and Moalli (1984) blocked a decrease in the number of perfused capillaries in the bullfrog web caused by air exposure with phenoxybenzamine, an α-adrenoceptor antagonist.

Significant control of CVR by cholinergic nerves is unlikely. Atropine had no effect on the responses to vagal stimulation, indicating the absence of cholinergic vagal control. Direct cholinergic control of CVR by nerves not associated with the vagus is also unlikely. Injections of acetylcholine into the perfusion line caused significant, but small (<10%), decreases in CVR. Thus, if cholinergic innervation of the cutaneous vessels exists, it probably has only a minor influence on CVR.

Although this study has described several aspects of possible autonomic regulation of CVR, the conditions under which such regulation may be used are still not known. Stimuli that alter cutaneous blood flow in amphibians include diving (Moalli *et al.* 1980), environmental hypoxia (Malvin and Hlastala, 1986*a*, 1989), environmental hypercapnia (Malvin and Hlastala, 1986*a*), lung ventilation (West and Burggren, 1984; Malvin and Hlastala, 1986*b*) and exposure to air (Burggren and Moalli, 1984). It is possible that autonomic control over CVR is involved in these changes of cutaneous perfusion but this remains to be tested.

This research was supported by NIH grant HL38942.

References

BOURGEOIS, P., DUPONT, W. AND VAILLANT, R. (1978). Estimation du taux catécholamines circulantes, chez la grenouille, à l'aide d'une technique de dosage radio-enzymatique. *C. R. hebd. Séanc. Acad. Sci. Paris* **287**, 1149–1152.

BURGGREN, W. AND MOALLI, R. (1984). "Active" regulation of cutaneous gas exchange by capillary recruitment in amphibians: experimental evidence and a revised model for skin respiration. *Respir. Physiol.* **55**, 279–392.

BURNSTOCK, G. (1969). Evolution of the autonomic innervation of visceral and cardiovascular systems in vertebrates. *Pharmac. Rev.* **21**, 247–324.

BURNSTOCK, G. AND BROWN, C. M. (1981). An introduction to purinergic receptors. In *Purinergic Receptors (Receptors and Recognition),* series B, vol. 12 (ed. G. Burnstock), pp. 1–45. London: Chapman & Hall.

CHRISTENSEN, C. (1974). Effect of arterial perfusion on net water flux and active sodium transport across the isolated skin of *Bufo bufo bufo* (L.). *J. comp. Physiol.* **93**, 93–104.

HERMAN, C. A., ROBLETA, D. O., MATA, P. L. AND LUJAN, M. D. (1986). Attenuated cardiovascular effects of prostaglandin I_2 and prostaglandin $F_{2\alpha}$ in cold acclimated American bullfrogs, *Rana catesbeiana. J. exp. Zool.* **238**, 167–174.

JONES, D. R. AND SHELTON, G. (1972). Factors affecting diastolic blood pressure in the systemic and pulmocutaneous arches of anuran amphibia. *J. exp. Biol.* **57**, 789–803.

KASAKOV, L. AND BURNSTOCK, G. (1983). The use of the slowly degradable analog, α,β-methylene ATP, to produce desensitisation of the P_2-purinoceptor: Effect on non-adrenergic non-cholinergic responses of the guinea-pig urinary bladder. *Eur. J. Pharmac.* **86**, 291–294.

KROGH, A., HARROP, G. A. AND REHBERG, P. B. (1922). Studies on the physiology of capillaries. III. The innervation of the blood vessels in the hind legs of the frog. *J. Physiol., Lond.* **56**, 179–189.

LANGLEY, J. N. (1911). The origin and course of the vaso-motor fibres of the frog's foot. *J. Physiol., Lond.* **41**, 483–498.

MAHANY, T. M. AND PARSONS, R. H. (1978). Circulatory effects on osmotic water exchange in *Rana pipiens*. *Am. J. Physiol.* **234**, R172–R177.

MALVIN, G. M. AND HLASTALA, M. P. (1986*a*). Regulation of cutaneous gas exchange by environmental oxygen and carbon dioxide in the frog. *Respir. Physiol.* **65**, 99–111.

MALVIN, G. M. AND HLASTALA, M. P. (1986*b*). Effects of intrapulmonary O_2, CO_2 and volume on cutaneous gas exchange in the frog. *Am. J. Physiol.* **251**, R941–R946.

MALVIN, G. M. AND HLASTALA, M. P. (1989). Effects of environmental O_2 on cutaneous blood flow and diffusing capacity in amphibians. *Respir. Physiol.* **76**, 229–242.

MOALLI, R., MEYERS, R. S., JACKSON, D. C. AND MILLARD, R. W. (1980). Skin circulation in the frog, *Rana catesbeiana*: distribution and dynamics. *Respir. Physiol.* **40**, 137–148.

PICK, J. (1970). *The Autonomic Nervous System. Morphological, Comparative, Clinical and Surgical Aspects*. pp. 215–239, Philadelphia, Toronto: Lippincott.

SEGURA, E. T., BRONSTEIN, A. AND SCHMAJUK. (1981). Effects of breathing upon blood pressure and heart rate in the toad, *Bufo arenarum* Hensel. *J. comp. Physiol.* **143**, 223–227.

SMITH, D. G. (1976). The innervation of the cutaneous artery in the toad *Bufo marinus*. *Gen. Pharmac.* **7**, 405–409.

SU, C. (1981). Purinergic receptors in blood vessels. In *Purinergic Receptors (Receptors and Recognition)*, series B, vol. 12 (ed. G. Burnstock), pp. 93–117. London: Chapman & Hall.

WAHLQVIST, I. AND CAMBELL, G. (1988). Autonomic influences on heart rate and blood pressure in the toad, *Bufo marinus*, at rest and during exercise. *J. exp. Biol.* **134**, 377–399.

WEST, N. G. AND BURGGREN, W. W. (1984). Factors influencing pulmonary and cutaneous arterial blood flow in the toad, *Bufo marinus*. *Am. J. Physiol.* **247**, R884–R894.

WILSON, J. X., BUTCHEY, J. K. AND DESHPANDE, A. A. (1988). Chicken gonadotropin-releasing hormone II increases plasma catecholamines in the bullfrog. *Neurosci. Lett.* **95**, 354–358.