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AUTONOMIC INFLUENCES ON HEART RATE AND BLOOD PRESSURE IN THE TOAD, *BUFO MARINUS*, AT REST AND DURING EXERCISE

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SUMMARY

Blood pressure (PA) and heart rate (HR) were measured in the conscious, resting toad, *Bufo marinus*. Treatment with bretylium (an adrenergic neurone blocking agent), alone or in combination with phentolamine and propranolol (adrenoceptor antagonists) did not alter PA or HR significantly. Atropine caused a small but significant increase in HR but had no effect on PA. The experiments indicate a cholinergic cardio-inhibitory tone but give no evidence for an adrenergic pressor tone at rest.

Treadmill exercise caused a rapid increase in PA and HR which was sustained throughout the exercise period. This response was partly psychogenic. The concentration of plasma catecholamines increased during exercise and was high enough to affect organs that were included in an extracorporeal blood circuit with the exercising animal. Bretylium treatment revealed an initial hypotension, presumably due to work hyperaemia, followed by a hypertension which was reduced compared to controls. The tachycardia was delayed but HR eventually reached control levels. Additional treatment with phentolamine and propranolol did not further affect the PA response, but significantly reduced the tachycardia reached during exercise.

It is concluded that the cardiovascular responses to exercise involve adrenergic nerve fibres causing hypertension and an initial rapid tachycardia. Circulating catecholamines seem to be the major cause of the sustained tachycardia.

INTRODUCTION

There have been many accounts of the autonomic innervation and responses to amines of parts of the cardiovascular system of anuran amphibians (see Burnstock, 1969; Nilsson, 1983). In particular, much information has been accumulated about the toad *Bufo marinus*. Physiological studies have covered the innervation of the

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heart (Kirby & Burnstock, 1969*b*; Morris, Gibbins & Clevers, 1981; Campbell *et al.* 1982) and of various vascular beds, e.g. lung (Campbell, 1971), kidney (Morris & Gibbins, 1983), spleen (Nilsson, 1978) and cutaneous artery (Smith, 1976). There have been histochemical studies of the neural distribution of catecholamines and peptides in other parts of the vasculature (McLean & Burnstock, 1966; Kirby & Burnstock, 1969*a*; Morris *et al.* 1985). Also, the catecholamine content of adrenal chromaffin and other tissues has been determined (Ungell & Nilsson, 1983). These studies give a good foundation on which to build an understanding of the circulation and its control in intact *Bufo*.

To date, there have been only a few *in vivo* studies of circulation in *Bufo* species. It has been shown that a baroreflex arising from the pulmocutaneous artery elicits cardioinhibition (Ishii & Ishii, 1978; Smith, Berger & Evans, 1981; Hoffman & Cordeiro do Souza, 1982; West & van Vliet, 1983) and pulmonary arterial constriction (West & Burggren, 1984), both mediated by cholinergic vagal fibres. It has also been found that both strenuous exercise (McDonald, Boutilier & Toews, 1980) and pulmonary inflation (Segura, Bronstein & Schmajuk, 1981) cause hypertension and tachycardia. Overall there is little indication of the part played by the autonomic nervous system in cardiovascular regulation. We have therefore used autonomic blocking drugs in an attempt to establish the role of autonomic nerves and of circulating catecholamines in the control of heart rate and blood pressure in conscious *Bufo marinus*, at rest and during exercise. Bretylium was used to block effects mediated by adrenergic nerves, followed by combined treatment with phentolamine and propranolol, antagonists of α- and β-adrenoceptors, respectively, to prevent actions of circulating catecholamines. Atropine was used, with or without prior adrenergic blockade, to prevent cholinergic nerve actions mediated by muscarinic receptors.

MATERIALS AND METHODS

Adult *Bufo marinus* weighing 70–140 g, captured in Queensland, Australia, were held for up to a month without feeding at 25°C and under a 12 h light:12 h dark regime. For all surgery and dissection, toads were anaesthetized by immersion in 0·3 % tricaine methanesulphonate (Rural Chemical Industries, NSW) adjusted to pH 7·0. All operated animals were killed by over-anaesthetization immediately after experimental observations had been made, i.e. about 30 h after the initial surgery.

Whole animal experiments

Surgical procedures

To record arterial blood pressure and heart rate, the left lateral aorta was cannulated non-occlusively with a polyethylene T-cannula introduced through a 1-cm dorsolateral incision immediately posterior to the left parotid gland. The cannula (i.d. 0·80 mm; o.d. 1·20 mm) was filled with 0·85 % NaCl containing heparin (50 i.u. ml^{-1}). The incision was closed with interrupted sutures and the cannula was stitched to the skin to prevent twisting. For injection of drugs, a saline-

filled polyethylene cannula (i.d. 0·80 mm; o.d. 1·20 mm) was introduced into the right iliac lymph sac through a small skin puncture and sutured into place.

In some animals central venous pressure was measured *via* an occlusive polyvinyl cannula (i.d. 0·80 mm; o.d. 1·20 mm) filled with heparinized saline, inserted into the ventral abdominal vein towards the heart through a 1-cm incision in the ventral midline.

Animals were allowed to recover for 20–24 h before recordings were made. Blood pressures were recorded using Statham P23 transducers linked to a Grass Model 79 polygraph. Instantaneous heart rate was measured from the arterial pressure pulse using a Grass 7P44 tachograph.

Experimental protocols

Experiments were made in a 25 °C room under dim lighting with a photoperiod similar to that of the holding room. While it was necessary to have one experimenter in the room to check that the cannula remained patent, disturbance was kept to a minimum.

Drug treatment. The relative importance of autonomic innervation and circulating catecholamines on the resting or exercise levels of blood pressure and heart rate was tested by using the following blocking agents. Bretylium tosylate, $10\,mg\,kg^{-1}$ (gift from Wellcome Laboratories, UK), was used to prevent release of adrenaline and noradrenaline from adrenergic nerves. It was given in two doses since the first dose causes a sympathomimesis that lasts for many hours in toads (Dumsday, 1975). We found in preliminary experiments that a second dose, given after the sympathomimesis had passed off, caused only brief increases in heart rate and mean arterial pressure. The first dose was given approximately 20 h before the start of the experiment and the second dose was given 1 h before the period chosen for observation or exercise.

Phentolamine mesylate, $2\,mg\,kg^{-1}$, and propranolol hydrochloride, $2\,mg\,kg^{-1}$ (Sigma), were used to block the effect of circulating catecholamines on α- and β-adrenoceptors, respectively, while muscarinic receptors were blocked with atropine sulphate, $2\,mg\,kg^{-1}$ (Sigma). All observations were made 1 h after drug administration. All injected drugs were dissolved in 0·1 ml of saline and 0·2 ml of saline was used for flushing in the drug.

Resting animals. Animals were prepared for recording of arterial pressure and heart rate as described above and kept in a closed translucent box, just large enough for them to turn around in. The box contained artificial pondwater to a depth of about 1 cm. Three groups of five animals were treated as follows. In the first group bretylium was given as previously described and blood pressure and heart rate were recorded (first observation period). Phentolamine and propranolol were then administered simultaneously and, following a 1-h equilibration period, HR and PA were recorded (second observation period). One hour prior to the final observation period atropine, in conjunction with phentolamine and propranolol, was injected. It was found necessary to re-administer the phentolamine and propranolol since their

action wore off with time. This protocol was used in an attempt to achieve a 'total autonomic blockade' (TAB). A second group was used to study the effect of blockade of muscarinic receptors with atropine. Vehicles of saline were injected 1 h before observations except for the last one where atropine was administered. The third group, control animals, was injected with saline vehicles at all times.

Exercising animals. Three groups of five animals were used and the protocols for drug treatment and animal instrumentation were identical to those used for the resting animals. Animals were held under a transparent Perspex cover placed over a treadmill. The cover was large enough to allow the toads to jump freely, and the treadmill was a Perspex drum with the outer walking surface roughened with fine sawdust glued to the surface. On rotation, the surface moved at 88 cm min^{-1} (approx. 10 body lengths min^{-1}). Toads commonly walked forward for 4–5 steps then paused until moved by the rotation back down the slope of the drum. The toads were exercised for 15-min periods 1 h following injections and were left undisturbed on the treadmill between observations.

'Startle' response. The operation of the treadmill involved the toad not only in a period of exercise but also in changes of visual and auditory input and of substrate vibration. These may have startled the animal and so may have elicited some of the responses described above. Three toads without drug treatment and three with total autonomic blockade were placed in a transparent box suspended from the frame of the treadmill about 5 cm above the surface. Arterial blood pressure and heart rate were recorded for 15 min when the treadmill was turned on. The same animals were then placed on the surface of the treadmill and, after 1 h of rest, subjected to exercise.

Isolated organ experiments

Paced atria

Toads were anaesthetized and opened ventrally. The left vagosympathetic trunk was freed from surrounding tissue between the heart and the vagal outflow from the skull. The sympathetic chain was then dissected free, great care being taken not to damage the fibres entering the vagus. The whole heart with attached vagosympathetic trunk and sympathetic chain was taken out and the ventricles were removed. Preparations were mounted for isometric force recording in an organ bath containing MacKenzie's solution (composition in mmol l^{-1}: NaCl, 115; KCl, 3·2; $CaCl_2$, 1·3; $MgSO_4$, 1·4; $NaHCO_3$, 20; NaH_2PO_4, 3·1; D-glucose, 16·7) at 25 °C, bubbled with 95 % O_2:5 % CO_2 to maintain a pH of 7·4. The atria were attached to a platinum hook and to a Grass FT-03 transducer and were electrically paced at 0·8–1·0 Hz (see Campbell *et al.* 1982). The nerves were placed on platinum ring electrodes shielded in plastic and the sympathetic chain and vagosympathetic trunk could be stimulated separately, using 30 s bursts of 1 ms, 15 V pulses at 2 Hz provided from a Grass S9 stimulator.

The same procedure was used for four preparations dissected from bretylium-treated animals 5 h after the second injection.

Bioassay experiments

In a further series of experiments, animals were prepared with an extracorporeal circuit for superfusion of assay organs. Both the anterior and posterior cut ends of the left lateral aorta were cannulated with polyethylene cannulae that were interconnected by a length of silicone tubing filled with heparinized saline, so that blood could flow from the anterior vessel and back into the posterior vessel. The wound was closed as above and the animal was allowed to recover from anaesthesia for 20 h. A second animal was then anaesthetized, about 2 ml of ventricular blood was drawn into a heparinized syringe, and either a pair of atria or the left hindlimb were used for bioassay. The toad was opened ventrally and the heart was excised. The ventricle was removed and the atria were mounted for force recording as described above. Alternatively, the left iliac artery was cannulated with polyethylene tubing (i.d. 0·50 mm, o.d. 0·90 mm) and the left hindlimb was removed from the body for perfusion. The silicone tubing connecting the two arterial cannulae of the exercising animal was cut and the ends were connected to two channels of a Watson–Marlow roller pump. Blood was pumped out of the anterior lateral aorta at 0·5–1·0 ml min^{-1} into a reservoir, initially filled with heparinized blood from the second animal, from which it was pumped back into the posterior lateral aorta. This blood circuit was used to superfuse the electrically paced atria which were mounted for force recording *via* a Grass FT-03 transducer. Alternatively, the blood was pumped through the hindlimb preparation *via* the iliac cannula and the arterial back pressure was recorded from the perfusion line with a Statham P23 pressure transducer; venous drainage was allowed to flow back into the reservoir for return to the host animal.

Blood analysis

Analysis of catecholamines

In a separate group of animals used for catecholamine assays, blood samples were collected from resting toads at least 30 min before the exercise period. A second sample was taken during the last minute of exercise. The samples (0·5 ml) were drawn from the arterial cannula into a clean syringe and were rapidly transferred to a plastic test tube containing 20 μl of an anticoagulant solution (EGTA, 95 mg ml^{-1}; glutathione, 60 mg ml^{-1}). The tube was centrifuged at 2°C (3000 rev. min^{-1}, 10 min) and the plasma was transferred to a clean tube, stoppered and stored at −20°C. Plasma adrenaline and noradrenaline levels were assayed radioenzymatically (Peuler & Johnson, 1977) with a sensitivity of 20 pg ml^{-1} of either amine in 100 μl of plasma.

Analysis of haematocrit

For determination of haematocrit, about 0·5 ml of blood was allowed to run freely from the arterial cannula into a plastic test tube. Two haematocrit capillaries were filled and the remaining blood was reinjected into the animal. The capillaries were centrifuged on a Hettich haematocrit centrifuge for 5 min at 25°C. Three samples were taken as follows: 30 min before exercise, in the last minute of exercise and

15 min after exercise. The same procedure was repeated in toads with total autonomic blockade. The effect of sampling was studied in control animals not undergoing exercise.

Analysis of data

Statistical analysis of data was performed by analysis of variance, except where otherwise stated, taking 5 % as the level of significance.

RESULTS

Validation of drug treatments

To check that the bretylium treatment produced the expected adrenergic neurone blockade, isolated atrial preparations were taken from animals 5 h after the second bretylium dose. In control electrically paced atria, stimulation of the sympathetic chain produced a positive inotropic effect while vagosympathetic nerve stimulation produced a negative inotropic response followed by a positive one. In preparations from bretylium-treated animals, sympathetic stimulation had no effect and vagosympathetic stimulation caused only negative inotropic responses.

Phentolamine and propranolol were used in combination as antagonists of α- and β-adrenoceptors, respectively, to prevent effects of circulating catecholamines. The doses used abolished responses of HR and PA to an intravenous dose of adrenaline ($1-2\,\mu g\,kg^{-1}$) which, if mixed in the total blood volume, would have given a plasma concentration of about $5\times10^{-8}\,g\,ml^{-1}$. This is about half the total catecholamine concentration in plasma taken from pithed (i.e. maximally stressed) *Bufo* (Ungell & Nilsson, 1983) and is about 10-fold greater than the highest concentration found in the present study. It therefore seems that the adrenoceptor blockade would have been adequate. However, full blockade of the adrenaline response could only be obtained for less than 2 h. Thus, to ensure a prolonged blockade, the phentolamine and propranolol were reinjected at the same time that atropine was given.

No direct test was made of the effectiveness of muscarinic blockade by atropine. However, several of the experimental animals showed a beat-to-beat variation in heart rate, presumably reflecting changes in vagal tone, that were eliminated by atropine treatment.

Since each of the drugs used seemed to work as expected, the animals treated with a combination of bretylium, phentolamine, propranolol and atropine will be described as having total autonomic blockade.

Animals under resting conditions

The experiments were performed in summer, the wet season in Queensland, when toads are active. In saline-treated animals in the first observation period, mean arterial pressure (PA) was $31{\cdot}7 \pm 1{\cdot}1\,cmH_2O$ ($1\,cmH_2O = 98{\cdot}1$ Pa) (mean ± S.E.M., $N = 10$) and heart rate (HR) was $23{\cdot}1 \pm 1{\cdot}8\,beats\,min^{-1}$ ($N = 10$). Similar values were obtained in the second and third observation periods (Fig. 1).

None of the drug treatments had any significant effect on resting PA (Fig. 1). Treatment with bretylium alone or in combination with phentolamine and propranolol had no significant effect on heart rate. In those animals treated with atropine alone, comparison of HR values before and after atropine (paired t-test) showed that HR was significantly increased by the treatment ($P < 0{\cdot}05$). However, comparison

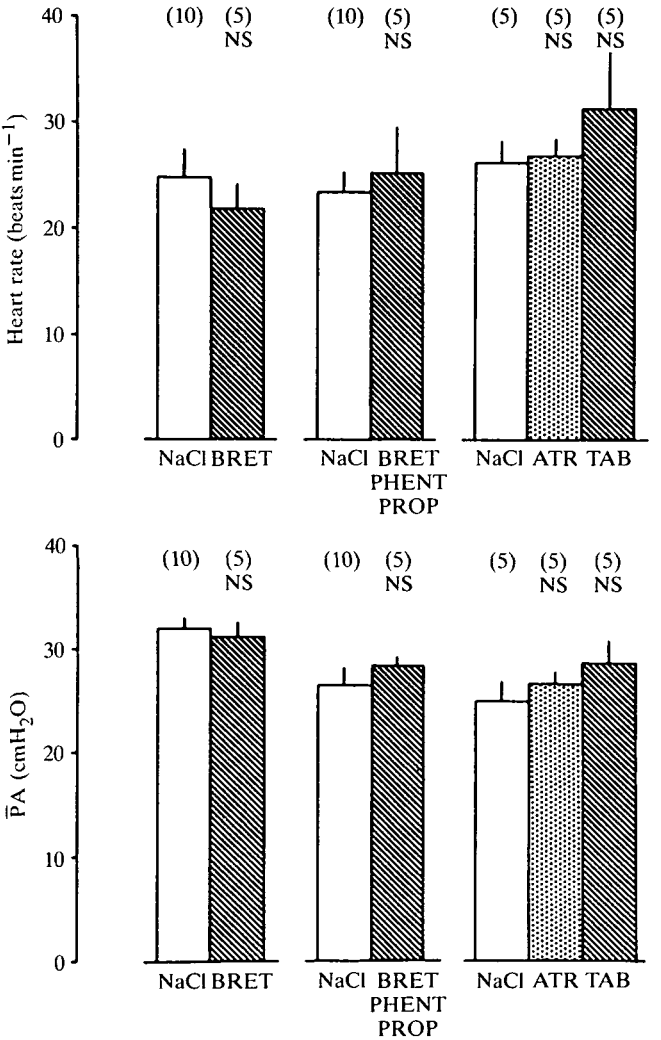

Fig. 1. Mean values of arterial blood pressure (PA) and heart rate (HR) in resting animals in the three observation periods following drug treatment. Hatched bars represent the group receiving total autonomic blockade (TAB, means ± S.E.M., $N = 5$), dotted bars represent animals with atropine (ATR) treatment (means ± S.E.M., $N = 5$). Saline-injected animals (clear bars) from the atropine group and controls were pooled in the first two observation periods (means ± S.E.M., $N = 10$). None of the drug treatments had any significant effect on resting PA. BRET, bretylium treatment; PHENT, phentolamine-treatment; PROP, propranolol treatment; NS, not significant.

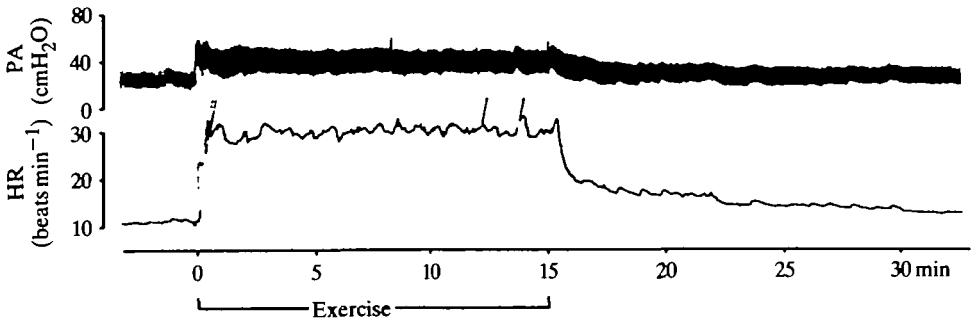

Fig. 2. Effects of exercise in a conscious toad on arterial blood pressure (PA) and heart rate (HR). Note the rapid increase in both pressure and heart rate, sustained during the 15-min exercise period.

between control- and atropine-treated groups at the last observation period showed no significant difference in HR.

Animals undergoing exercise

These experiments were also carried out in the summer months. When saline-injected animals were first exercised, they showed a rapid increase in both HR and PA (Fig. 2). The values of PA essentially reached a maximum within 1 min of the onset of exercise and remained at that level for the rest of the 15-min exercise period, while HR reached a steady level after about 2 min. PA was increased by about 15 cmH_2O and HR rose by about 20 beats min^{-1}. At the end of the exercise period, both HR and PA fell to pre-exercise levels over 15–30 min (Fig. 2).

Similar responses occurred in saline-treated animals in the second and third exercise periods. But although the increase of PA by the end of the exercise period was nearly the same in each period, the rise of PA at the beginning of exercise became progressively slower. Whereas in the first period the value of PA after 1 min was about 100 % of the maximum, in the second and third period it was only 75 % and 60 %, respectively, of the maximum reached in those periods. The rate of increase of HR may also have been slightly slower in the second and third periods (Figs 3, 4).

Bretylium treatment significantly slowed the rate of increase of HR at the beginning of exercise (Figs 3, 4). It also reduced the level of HR that was sustained until the end of exercise, but this effect was not statistically significant. In addition, the response of PA to exercise was changed in form: hypotension now occurred at the

Fig. 3. Mean values of arterial blood pressure (PA) and heart rate (HR) in five blocked (broken lines) and five control toads (solid lines). Bars represent S.E.M. (A) Effects of bretylium treatment on changes in blood pressure and heart rate during the first exercise period. Note the slow increase in heart rate in the blocked animals at the onset of exercise. (B) Effect of treatment with bretylium and the α- and β-antagonists phentolamine and propranolol on the response to exercise. Note the increase in blood pressure after the exercise period. (C) Effects of total autonomic blockade (bretylium, phentolamine, propranolol and atropine) on the response to exercise.

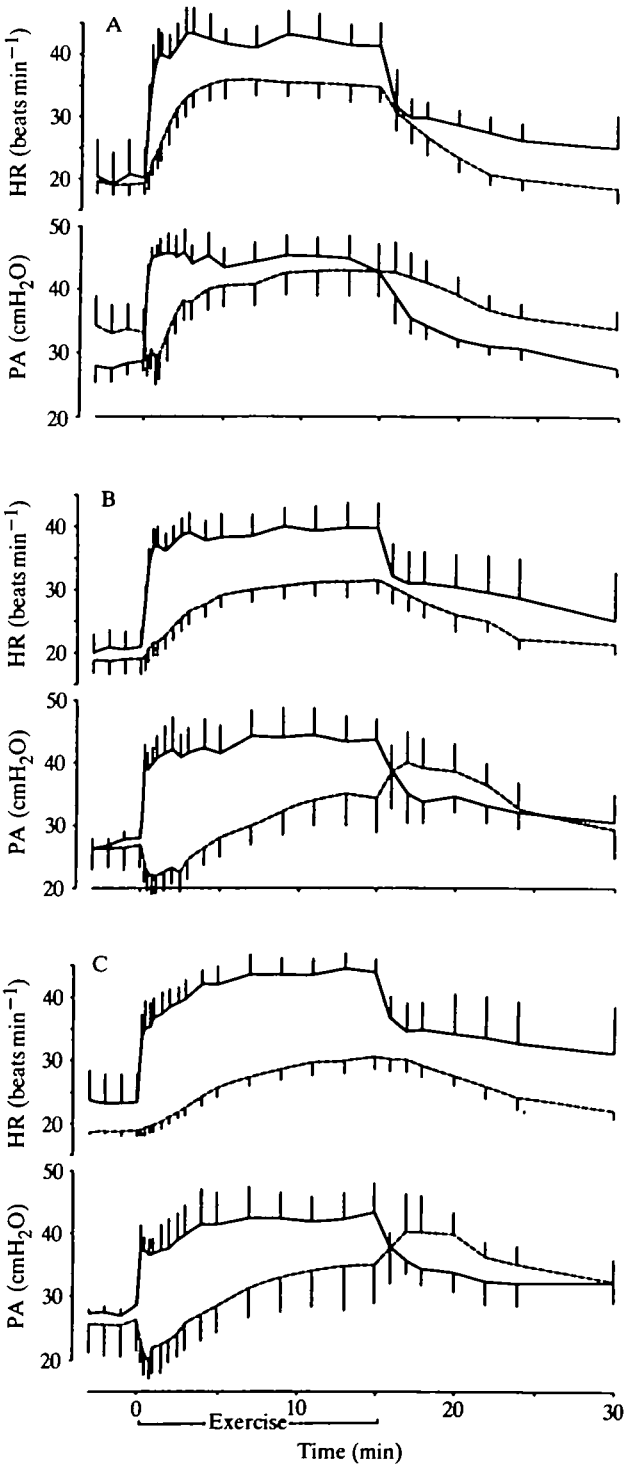
A
B
C
HR (beats min^{-1})
PA (cmH$_2$O)
40
30
20
50
40
30
20
0
10
20
30
Exercise
Time (min)

Fig. 3

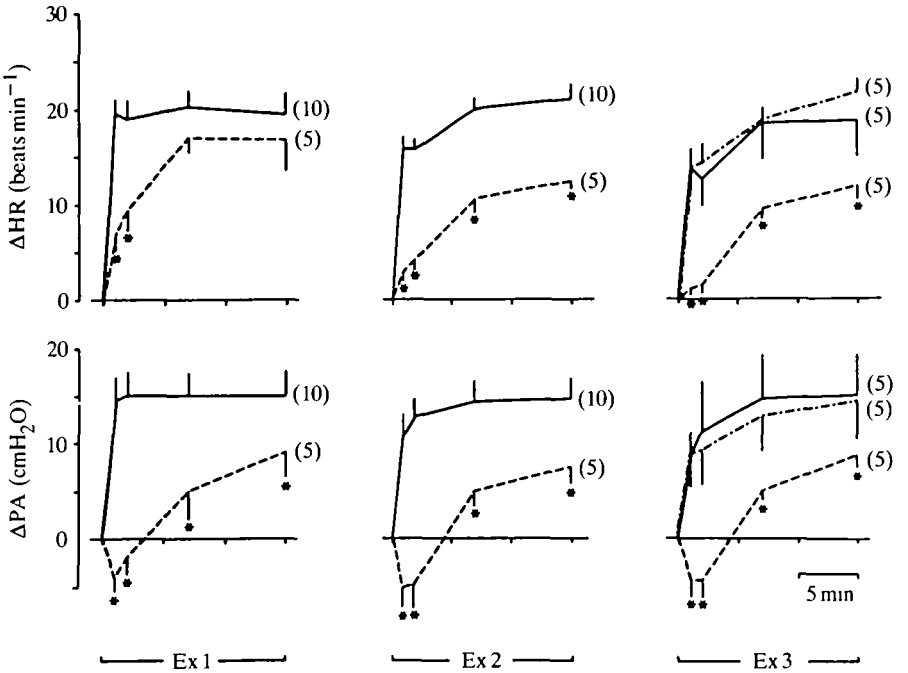

Fig. 4. Mean values of the changes in arterial blood pressure (ΔPA) and heart rate (ΔHR) after 1, 2, 7 and 15 min of exercise. Left panel: comparison between bretylium-treated animals (broken lines, $N = 5$) and saline-injected animals (solid lines, $N = 10$) during the first exercise period (Ex 1). Bretylium treatment significantly reduces ΔHR in the first minutes of exercise but control levels are eventually reached. ΔPA is significantly reduced by bretylium during the whole exercise period. Middle panel: comparison between animals with adrenergic blockade with bretylium, phentolamine and propranolol (broken lines, $N = 5$) and saline-treated animals (solid lines, $N = 10$). Additional treatment with phentolamine and propranolol prior to the second exercise period (Ex 2) caused a further reduction of ΔHR but no further change in ΔPA. Right panel: comparison between animals with total autonomic blockade (broken lines, $N = 5$), atropine-treated animals (semi-broken lines, $N = 5$) and saline-treated animals (solid lines, $N = 5$) in the third period of exercise (Ex 3). Atropine alone or in combination with adrenergic blocking drugs does not significantly affect the response to exercise (analysis of variance, $P < 0{\cdot}05$). Asterisks signify significant differences compared with controls ($P < 0{\cdot}05$).

beginning of exercise, reaching a maximum in about 30 s (Figs 3, 4). PA then started to increase, reaching pre-exercise levels after about 90 s of exercise. After about 5–10 min of exercise, PA reached a sustained level that was lower than in saline-treated animals but not significantly so. However, the pre-exercise level of PA was rather higher in saline-treated animals, so that the increase of PA (ΔPA) caused by exercise was significantly reduced by bretylium. At the end of the exercise period, PA fell to pre-exercise levels more slowly than it did in saline-treated animals (Fig. 3), while HR fell, if anything, slightly more rapidly.

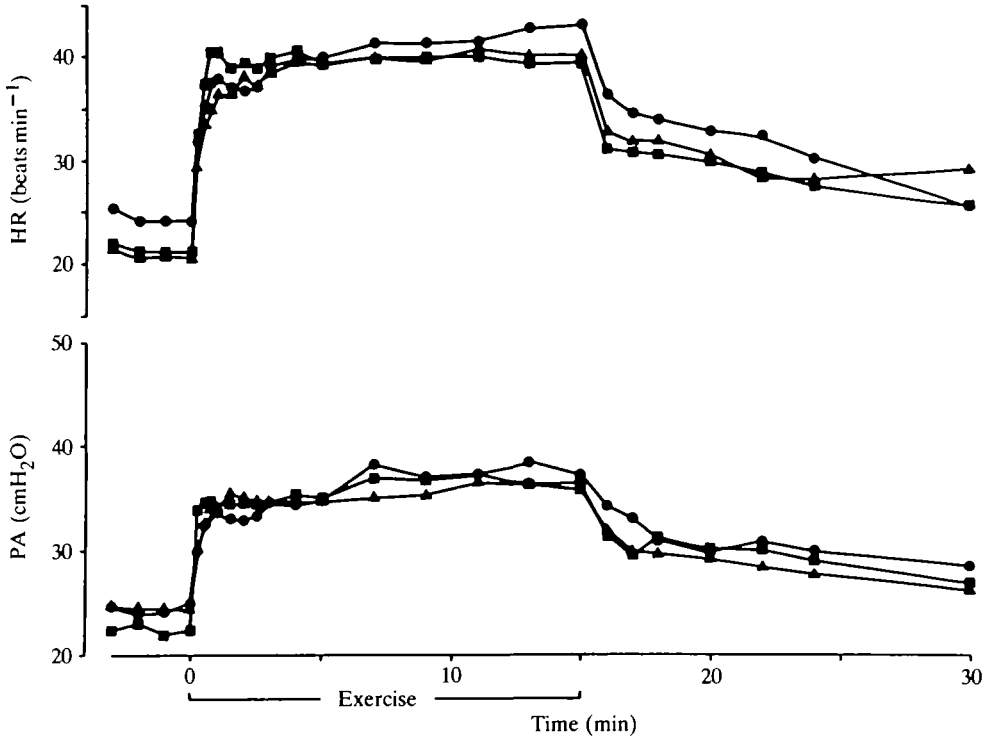

Fig. 5. Mean values of arterial blood pressure (PA) and heart rate (HR) in the three exercise periods in the atropine-treated group. Exercise 1, saline-treated (squares); exercise 2, saline-treated (triangles); exercise 3, atropine-treated (circles). $N = 5$ for all points.

Additional treatment of bretylium-treated animals with phentolamine and propranolol did not change the hypotension at the beginning of exercise nor did it significantly reduce the maximum ΔPA in exercise (Fig. 4). However, the absolute PA reached during exercise was then significantly less than in saline-treated animals (Fig. 3). Furthermore, after the end of the exercise period, PA rose for several minutes before falling towards pre-exercise levels. In addition, the rise of HR during exercise was significantly smaller than in saline-treated animals, although the rate of recovery after exercise seemed to be unchanged.

Atropine, whether given alone or after treatment with bretylium, phentolamine and propranolol, produced no significant change in the existing responses to exercise or the recovery after exercise (Figs 4, 5). It is interesting to note that in the animals with total autonomic blockade, exercise still caused PA to rise by about 9 cmH_2O and HR to rise by 12 beats min^{-1} (Figs 3C, 4). In six animals not treated with drugs, central venous pressure was recorded during exercise. Movements of the animal often twisted the cannula and occluded the thin-walled ventral abdominal vein, preventing recording. In two animals in which the cannula appeared to remain patent during exercise, central venous pressure was about 4 cmH_2O at rest. At the

Table 1. *Haematocrit (Hct) and catecholamine content in blood from cannulated conscious toads*

	Test period		
Rest	1	2	3
Hct (%)	17·2 ± 1·50	15·9 ± 1·50	15·0 ± 2·00
Exercise	Before	During	After
Hct (%) Controls	13·3 ± 1·50	12·5 ± 0·90	12·8 ± 1·60
Hct (%) Total autonomic block	17·8 ± 0·90	16·5 ± 1·20	15·9 ± 1·10
Exercise	Before	During	
Catecholamines			
Controls			
Adrenaline ($ng\,ml^{-1}$)	2·30 ± 0·30	4·80 ± 0·50	
Noradrenaline ($ng\,ml^{-1}$)	0·06 ± 0·02	0·30 ± 0·05	

The figures represent means ± S.E.M. from three animals.

onset of exercise, venous pressure rose to about 8 cmH_2O within 30 s and remained high throughout the period of exercise.

There was considerable variation of haematocrit (Hct) between animals (mean resting values 15%; range 11–20%). When three sequential blood samples were taken from resting animals, Hct fell progressively but not significantly (Table 1). Animals with total autonomic blockade and saline-treated controls were sampled before, at the end of, and 15 min after a 15-min exercise period; they showed a similar non-significant fall of Hct over the three sample periods and no specific effects of exercise could be detected.

Other animals without drug treatment were used for the assay of plasma catecholamines, sampled before and in the last minute of a 15-min exercise period (Table 1). By the end of the exercise period, adrenaline concentrations had risen about two-fold and noradrenaline levels about five-fold.

Experiments with an extracorporeal blood circuit

Eight animals were operated on to produce an extracorporeal blood circuit which was used to supply an assay organ. In four cases the assay organ was a superfused, electrically paced atrial preparation taken from a toad treated with 6-hydroxydopamine ($50\,mg\,kg^{-1}$), given to produce 'denervation supersensitivity' to catecholamines. Three preparations showed no response to exercise of the host animal. The fourth preparation showed a repeatable increase in force of contraction when the host was exercised (Fig. 6). The response was abolished when phentolamine and propranolol, each $2\,mg\,kg^{-1}$ host, were added to the blood reservoir.

In four animals, the extracorporeal blood was used for constant-flow perfusion of the vasculature of an isolated hindlimb preparation. Two of the preparations showed a repeatable increase in perfusion back pressure when the host was exercised (Fig. 7). The two other preparations showed no responses.

Startle responses

Three toads without drug treatment were placed in a transparent box suspended from the frame of the treadmill about 5 cm above the surface. When the treadmill was started, HR and PA rose rapidly: after 1 min, the values were similar to those seen in exercising animals (Fig. 8). However, both HR and PA then fell progressively throughout the rest of the 15-min period of operation of the treadmill, reaching values only slightly above pre-exercise levels. The same animals were then placed on the surface of the treadmill and, after 1 h rest, subjected to exercise; they showed increases of HR and PA that were sustained throughout the period of exercise (Fig. 8).

Similar experiments were carried out on three animals subjected to total autonomic blockade. When suspended above the treadmill, they showed no cardiovascular responses to its operation. When exercised on the treadmill they showed responses like the blocked animals described above (Fig. 9).

DISCUSSION

The adrenergic neurone-blocking drug bretylium was shown to prevent sympathetic effects on the heart. In *in vitro* experiments, bretylium has been shown to block adrenergic neurotransmission to all major cardiovascular effectors examined in *Bufo*: the heart (Morris *et al.* 1981) and the pulmonary (Campbell, 1971), cutaneous (Smith, 1976), renal (Morris & Gibbins, 1983), skeletal muscle and mesenteric

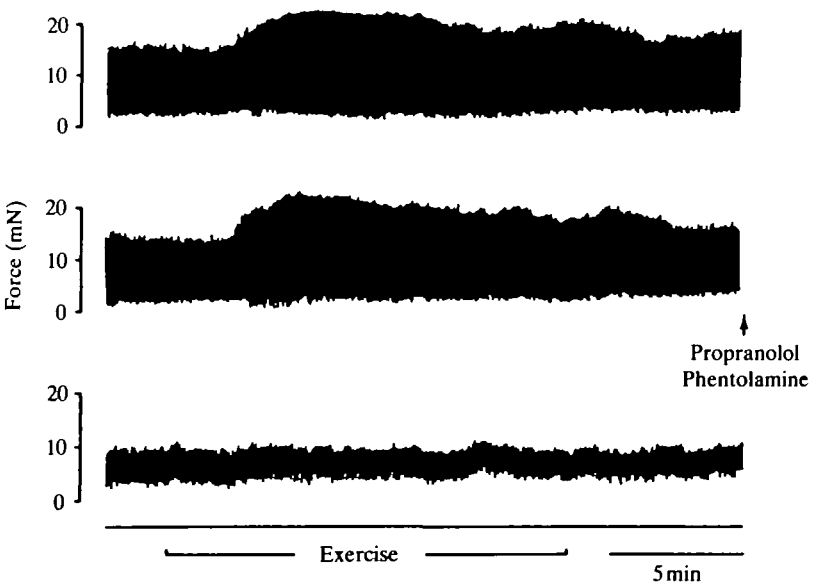

Fig. 6. Electrically paced toad atria superfused with blood from a conscious host animal *via* an extracorporeal blood circuit. The two upper panels show changes in the force of contraction induced by repeated exercise by the host animal. The response is abolished after blockade of the adrenergic receptors (lower panel).

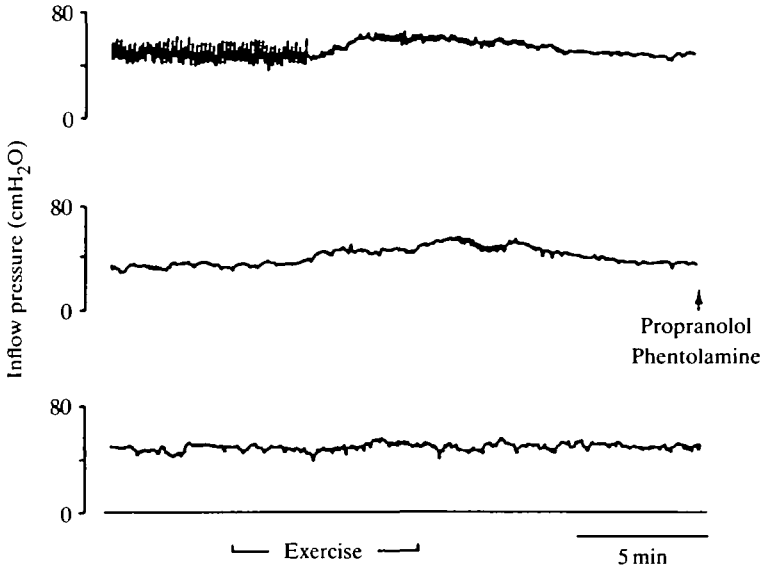

Fig. 7. Toad hindlimb perfused with blood from a conscious host animal *via* an extracorporeal blood circuit. The two upper panels show changes in inflow pressure induced by repeated exercise by the host animal. The response is abolished after blockade of the adrenergic receptors. The spikes in the first pressure trace are due to pulsations caused by the roller pump. They were later electrically dampened on the recorder.

(Dumsday, 1975) vascular beds. Furthermore, adrenergic neurone blockade seems to prevent the release from adrenergic nerves not only of catecholamines but also of other active substances, e.g. neuropeptide Y (Lundberg, Änggård, Theordorrson-Norheim & Pernow, 1984) and adenosine triphosphate (see Fedan *et al.* 1981). It is therefore likely that the bretylium treatment did prevent all cardiovascular effects mediated by adrenergic nerves.

It is likely that atropine abolished all cholinergic transmission to the heart and vasculature. Some minor actions of the autonomic nervous system, e.g. the inhibitory action of somatostatin on the heart (Campbell *et al.* 1982) and other peptide-mediated actions on the vasculature, would not have been antagonized.

There do not appear to have been unwanted side effects of the drugs. For instance, bretylium has muscarinic antagonistic properties (Schreiber, Friedman & Sokolovsky, 1984) and can inhibit cholinergic transmission to the heart *in vitro* (Smith, Nilsson, Wahlqvist & Eriksson, 1985), but vagal cardio-inhibition in the toad was not blocked by bretylium in the present experiments.

Animals at rest

The resting animals used in this study seem to have been only minimally disturbed. Dumsday (1975) measured a HR down to 15 beats min^{-1} in unoperated, conscious *Bufo marinus*, instrumented with ECG leads. HR became progressively lower as the animals were held in more secluded surroundings. In the present

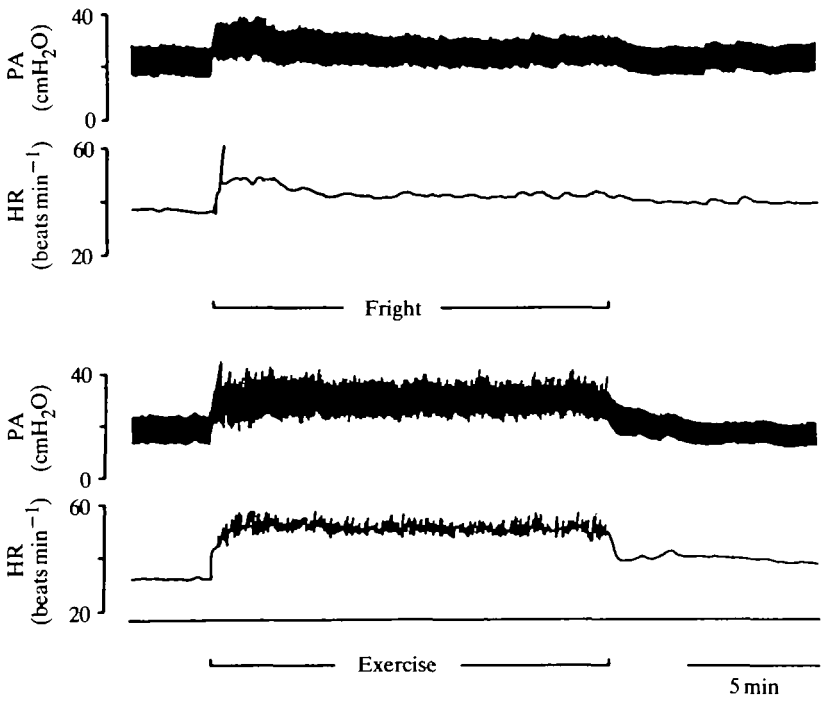

Fig. 8. Startle response from an untreated conscious toad placed in a transparent box suspended from the frame of the treadmill 5 cm above the surface. The upper panel shows changes in arterial blood pressure (PA) and heart rate (HR) when the treadmill was turned on for 15 min. The lower panel shows the effects on PA and HR when the same animal was exercised.

experiments HR was about 23 beats min^{-1}, considerably lower than the resting levels reported in other studies of this species (e.g. McDonald *et al.* 1980; Smith *et al.* 1981; Segura *et al.* 1981).

The various drug treatments had no significant effects on mean HR and PA in resting toads. However, atropine slightly increased HR and eliminated the beat-to-beat variations in HR. The results imply that cholinergic vagal fibres do restrain HR slightly but that the unrestrained HR is within the normal range for untreated resting animals. A simple interpretation of the results is that the cardiovascular system in resting *Bufo* is not under autonomic control. But this obvious conclusion is not necessarily true. It is conceivable that there is, in fact, an autonomic constrictor tone in the resting toad but that the PA that it produces is the same as that produced by myogenic autoregulation when autonomic influences are removed.

Responses to exercise

Untreated animals

Exercise in unblocked toads caused rapid and sustained increases in HR and PA, as already reported for this species (McDonald *et al.* 1980; Boutilier, McDonald &

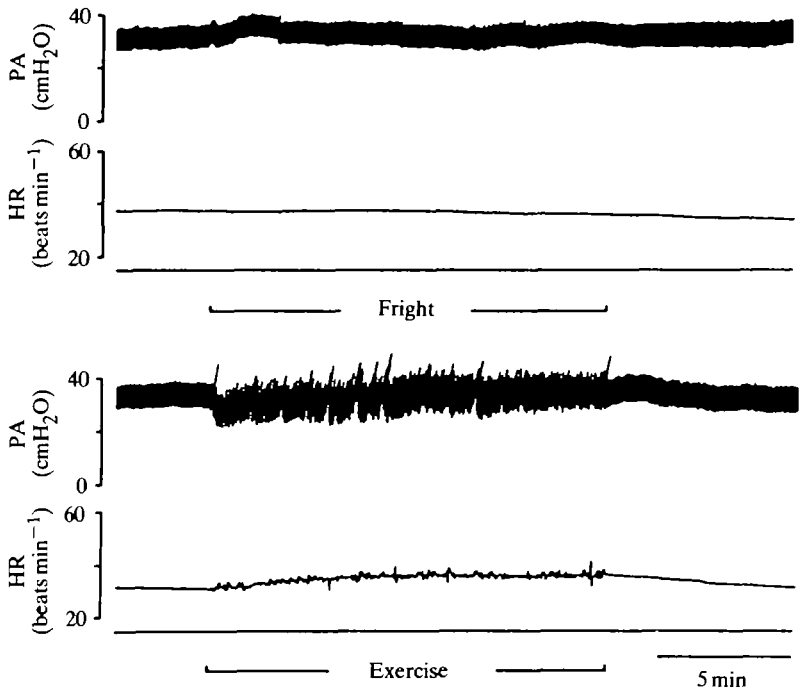

Fig. 9. Startle response from a conscious toad with total autonomic blockade. The upper panel shows the effects on PA and HR when the treadmill was turned on for 15 min. The lower panel shows the same blocked animal during exercise.

Toews, 1980). Discussion of autonomic participation is difficult because animals with total autonomic blockade still showed sizeable responses, some of which might not occur in unblocked animals. A qualitative interpretation of the results of blockade is that circulating catecholamines are involved in the sustained increases of HR and PA, and that adrenergic nerves are involved in the cardiovascular responses especially over the first few minutes of exercise. Since atropine, given alone or after adrenergic blockade, had no effect on the pre-existing cardiovascular responses to exercise, cholinergic nerves probably play no major role.

By the end of the exercise period there was an increase in the level of adrenaline in the plasma, and an even greater rise in noradrenaline level. Stress causes a similar change in the plasma ratio of the two amines in *Rana esculenta* (Bourgeois, Dupont & Vaillant, 1978). The amounts released in exercising *Bufo* were sufficient to affect both cardiac and vascular preparations put into an extracorporeal blood circuit as assay organs. In animals with adrenergic neurone blockade, the most obvious effect of additional α- and β-adrenoceptor blockade was a decrease in the plateau levels that both HR and PA reached after several minutes of exercise. While the hypertension might reflect only an increase in cardiac output, it is probable that the amines also caused peripheral vasoconstriction since that response was seen in hindlimb beds included in an extracorporeal circulation. Thus, since both chemical and biological

assays showed the release of catecholamines during exercise in unblocked animals, it is probable that the amines are normally involved.

Judging from the effects of bretylium treatment, adrenergic nerves mediate the rapid increases in HR and PA that occur at the onset of exercise; in treated animals the rise of HR was slower than normal and hypotension replaced the normal initial hypertension. The early hypertensive effect attributable to adrenergic nerves might reflect just an increase in cardiac output, but there may also have been an adrenergic vasoconstriction. There is less evidence that the adrenergic nerves are involved in the cardiovascular changes seen later in the exercise period. The plateau level of heart rate in continued exercise was only slightly reduced in bretylium-treated animals and the absolute PA attained also did not change significantly. But, if only because pre-exercise levels of PA were somewhat high, bretylium treatment significantly reduced the increment of PA during exercise. Overall it appears that the sympathetic nervous effects on the heart and probably on the vasculature increase rapidly at the onset of exercise and fade as exercise continues. The pattern resembles the changes in HR and PA seen when animals were suspended over the moving treadmill without walking. These startle responses were abolished by total autonomic blockade and are most readily ascribed to the actions of sympathetic nerves, although we did not prove this point by testing the effects of bretylium alone. It seems probable that most of the apparently neurogenic cardiovascular changes during exercise are startle responses and are not related to exercise. In fact, the rise of HR and PA at the onset of exercise became progressively slower in control animals as they were subjected to repeated periods of exercise: it appears that the toads became accustomed to the operation of the treadmill, which supports the idea that the initial cardiovascular responses may have been predominantly psychogenic.

Blocked animals

In animals with total autonomic blockade, exercise induced a hypotension that was rapid in onset, followed by a slowly developing increase in HR and PA. After exercise, HR fell but PA actually rose further for some minutes before declining.

The initial hypotension was revealed by adrenergic neurone blockade and was not changed by further blockade of adrenergic or muscarinic receptors. A similar hypotension occurs at the start of exercise in mammals that have had cardiac sympathetic denervation (Donald & Shepherd, 1964) or have been treated with adrenoceptor antagonists (Bassenge, Kucharczyk, Holtz & Stoian, 1972; Bassenge, Holtz, v. Restorff & Oversohl, 1973). The most likely cause of the hypotension is a decrease of peripheral resistance caused by metabolic vasodilatation in working muscles. In mammals, such vasodilatation begins within 0·5 s of the onset of exercise (Donald & Shepherd, 1963; Bevegard & Shepherd, 1967). It is an autoregulatory process of local origin and is directly related to the metabolic need of the muscle (Ceretelli, 1966). In fact, work hyperaemia in amphibian muscle was described long ago (Krogh, 1918; Krogh, Harrop & Rehberg, 1922). A second factor that might be involved is the phasic emptying of intramuscular veins by repeated muscle contraction (Folkow, Gaskell & Waaler, 1970), an effect that would be expected to

increase muscle perfusion by steepening the pressure gradient along muscle capillaries. Although the hypotensive component first became apparent in animals with adrenergic blockade, it was probably present in untreated animals but masked by adrenergic pressor effects; the rise in central venous pressure seen in untreated animals was most probably caused by muscle vasodilatation shifting blood to the venous system.

There are several additional changes occurring in exercise that might initially increase venous return by decreasing venous volume. These include compression of major abdominal veins caused by an elevation of intra-abdominal pressure in response to postural contraction of abdominal muscles and inflation of the lungs. Segura *et al.* (1981) have shown that abdominal contraction and lung inflation, as well as intravenous saline injection, induce tachycardia and hypertension in *Bufo arenarum*; the effects are mediated only partly by the autonomic nervous system, suggesting that the stimuli can cause an autoregulatory increase in cardiac output. The increase in HR seen in blocked exercising animals most probably reflects such autoregulation.

The cause of the hypertension that developed during exercise in blocked animals is not known. Part of the hypertension might be caused directly by increased venous return leading to increased cardiac output. But the changes of venous return were presumably stable after the first few minutes of exercise, whereas the blood pressure continued to increase throughout most of the exercise period. The present experiments with blood-bathed assay organs placed in the circulation did not reveal the release of a non-adrenergic vasoconstrictor or cardiac stimulant during exercise. The possibility was considered that exercise might increase blood viscosity, so promoting hypertension, but no systematic increase of Hct was found. The most remarkable feature of the hypertension was that, for several minutes after exercise, PA was actually higher in blocked than in unblocked animals (Fig. 3C). This suggests that the mechanism underlying the hypertension seen in blocked animals is either absent or has only a minor role in unblocked animals. To account for this, and in a spirit of pure speculation, a model is proposed for the exercising blocked animal. First, it is proposed that exercise initiates vasodilatation in the active skeletal muscles *via* metabolic autoregulation, causing hypotension as blood is passed to the venous system. The increased venous return would increase HR and presumably cardiac output by cardiac autoregulation, producing a return of PA towards pre-exercise levels. In addition, it is suggested that the abdominal veins are emptied by increased lung inflation and by postural tone in abdominal muscles. This would further increase cardiac output, causing PA to exceed pre-exercise levels. In the blocked animal this rise in pressure would elicit myogenic autoregulation, increasing peripheral resistance. (No such response occurs in the untreated animal, because the terminal vasculature is protected by autonomic constriction of proximal arteries). The increase in resistance would increase PA, eliciting further myogenic vasoconstriction. The positive feedback between precapillary resistance and PA would continue until a new higher equilibrium level of PA is reached. It would be the myogenic vascular autoregulation, occurring in all vascular beds not undergoing

metabolic autoregulation, that would constitute the non-autonomic hypertensive component to the response to exercise. At the end of exercise the muscular metabolic vasodilatation would pass off rapidly, causing an additional hypertension. Then, as blood is moved from the arteries back to the no longer compressed abdominal veins, the feedback between myogenic vasoconstriction and PA would slowly bring PA back to its normal state.

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