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Cardiovascular effects of toxins isolated from the cnidarian *Chironex fleckeri* Southcott

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Summary

1. Two unstable high molecular weight toxins have been isolated from tentacles of *Chironex fleckeri* by exclusion chromatography. Both are cardiotoxic ; the lower molecular weight fraction is also a potent haemolysin.

2. Both toxins reduce the rate, amplitude of contraction and coronary flow in the isolated, perfused guinea-pig heart. Relative to the mouse lethal dose the haemolytic fraction is less potent in this preparation than the purely cardiotoxic fraction.

3. Both toxins cause a rise in arterial pressure in anaesthetized rats and rabbits by a direct action on the vascular musculature. This is followed by hypotension, bradycardia and cardiac irregularity. An increase in respiratory rate is followed by apnoea of variable duration, which is associated with a rise in arterial pressure. Animals frequently show arterial pressure oscillations with periods of apnoea interspersed with hyperpnoea.

4. The carotid occlusion reflex is depressed during hypotensive periods after both toxins, although (−)-noradrenaline can still elicit a marked pressor response. Bilateral cervical vagotomy has but little effect on the response to either toxin, save to prevent hyperpnoea, but radical denervation of sinoaortic afferents reduces the arterial pressure fall after the initial hypertensive response, suggesting that this fall is due to a combination of baroreceptor stimulation and a fall in cardiac output. Blood pressure oscillations are still seen, possibly due to central stimulation by hypercapnia.

5. Interference with the efferent arm of the vasomotor reflex arc with hexamethonium, bretylium or phenoxybenzamine either abolishes or markedly reduces the blood pressure oscillations without affecting the initial hypertensive response.

6. The cardiovascular effects of the two toxins are thought to be due to direct vasoconstriction, cardiotoxicity, baroreceptor stimulation and possibly depression of the vasomotor centre. The resultant disordering of the feed-back system regulating vasomotor tone leads to the characteristic arterial pressure oscillations.

Introduction

The clinical picture of death from stinging by the box jellyfish, *Chironex fleckeri* Southcott (Barnes, 1966) correlates well with the findings in experimental animals given injections of toxins isolated from the tentacles (Freeman & Turner, 1969). The

cause of death in animals was respiratory arrest of central origin, associated with cardiovascular failure. Death occurred in less than 2 min after high doses of the toxin preparation but could be delayed for upwards of 30 min after lower doses. In such cases the animals showed marked arterial pressure oscillations before death.

The toxin consists of at least two high molecular weight fractions (Crone & Keen, 1969) with differing pharmacological properties. The present paper is concerned with a study of the cardiovascular effects of these two fractions in the rat and the rabbit. While both fractions have cardiotoxic properties, only the 70,000 molecular weight fraction is haemolytic. Relative to the 'mouse lethal dose' this fraction, which for convenience has been termed the 'haemolysin', has less cardiotoxicity than the heavier (approximately 150,000 molecular weight) 'cardiotoxic' fraction.

Our chief concern has been to investigate the cardiovascular effects after minimal lethal doses, since it is only those victims which survive for about 15–20 min after stinging that are likely to receive medical attention in time for treatment to be effective.

Methods

Preparation of toxins

Frozen tentacle was homogenized by grinding with a pestle and mortar containing liquid nitrogen. A saline solution (150 mM NaCl buffered to pH 8 with 5 mM Tris) was added to the mortar in the proportion of 1 ml saline to 400 mg tentacle. The debris was centrifuged down before 3 ml of the supernatant were placed on a Sephadex G200 column at 5° C. The solution contained 10,000 mouse units (M.U.) per ml. Fractions of volume 4 ml were collected over 48 h and stored at −20° C. The lethality of the fractions was determined by injection, after suitable dilution, of 0·1 ml into the lateral tail veins of albino mice weighing 25–30 g. The haemolytic activity was determined by measuring the time required for complete haemolysis of 1 ml of a standard red cell suspension by 0·1 ml of the toxin fraction, when incubated at 25° C and pH 7·4 (Crone & Keen, 1969).

Figure 1 shows that two well defined lethal fractions separated on Sephadex filtration ; the second fraction to pass through the column contained all the haemolytic activity. For the pharmacological investigations only the peaks of these fractions corresponding to elution volumes from 75 to 90 ml, and 105 to 115 ml were used. The area under the lethality curve for each fraction consistently suggested that the cardiotoxic fraction contained the greater part of the lethality of the original extract. This may, however, be due to the instability of the toxins at 5° C, since the haemolytic fraction took longer to pass through the column than the cardiotoxic fraction.

Pharmacological testing of toxins

The effects of the two toxins on arterial pressure, right atrial pressure, rate and depth of respirations, electrocardiogram and heart rate were determined using Wistar rats (220–300 g) and New Zealand rabbits (2–3 kg), as was described previously (Freeman & Turner, 1969). Rabbits were anaesthetized with urethane and

maintained with chloralose ; rats were anaesthetized with pentobarbitone (40–50 mg/kg) given by a hypodermic needle which was held in a lateral tail vein with adhesive tape.

When it was intended to investigate baroreceptor reflexes the femoral rather than the carotid artery of the rabbit was cannulated. In some cases the nerves to the aortic and carotid baro- and chemoreceptors were acutely sectioned in the rat by careful dissection of the cervical region with the aid of a magnifying loupe. The superior laryngeal, the recurrent laryngeal, aortic depressor and vagus nerves and the sympathetic trunk were sectioned, as well as the nerves arising in the carotid bifurcation (Krieger & Marseillan, 1963).

The intravenous LD50 for both toxins was determined in mice using the tables of Weil (1952). However, because of the instability of the toxins, the MLD for groups of four to five mice was determined daily immediately before use. Death within 5 min was arbitrarily used to estimate the MLD for both fractions.

The effect of the toxins on the isolated perfused guinea-pig heart was determined as described previously (Turner & Freeman, 1969). Heart rate, coronary flow and isotonic contractions were recorded simultaneously.

Drugs used were hexamethonium bromide, (−)-noradrenaline bitartrate, bretylium tosylate, phenoxybenzamine hydrochloride and propranolol hydrochloride.

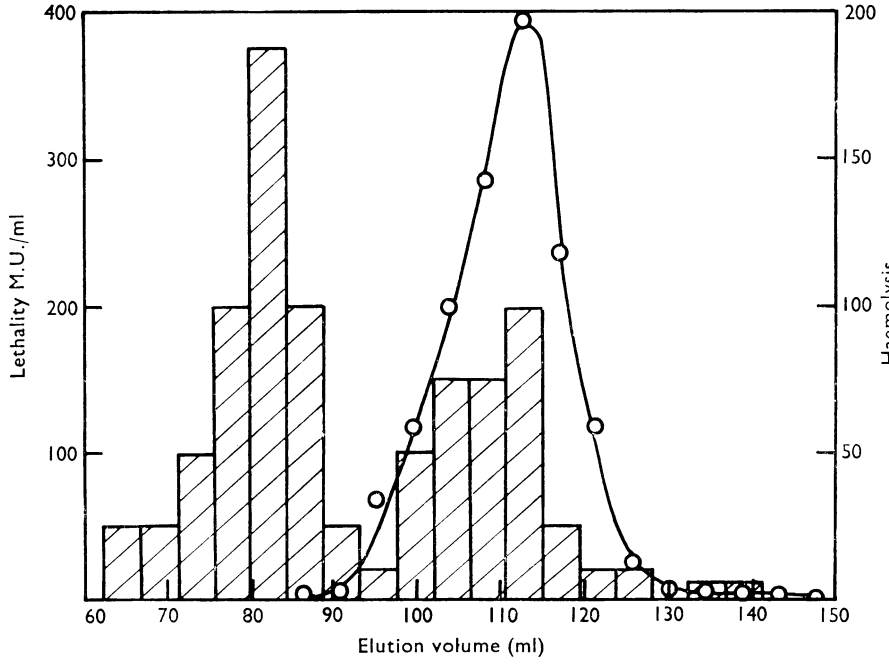

FIG. 1. Separation of the cardiotoxic and haemolytic fractions by exclusion chromatography on Sephadex G-200 in 150 mM NaCl, 5 mM Tris, pH 8·0. The void volume as defined by the dextran blue marker was 65 ml. Haemolytic activity (○——○) is expressed as $\frac{1}{\text{relative dilution}} \times 10^3$ (Crone & Keen, 1969).

Results

Acute toxicity

Death in mice after intravenous injection of either toxin showed a similar pattern to that described for the crude extract, or for ' amnion-milked ' toxin obtained from Dr. Barnes (Barnes, 1967 ; Freeman & Turner, 1969). The dose-mortality curve was steeper for the cardiotoxic fraction than for the haemolytic fraction, and the onset of effects was more rapid. At autopsy the mice all showed marked venous engorgement, with a variable degree of staining of the lungs. The heart was usually still beating feebly and irregularly.

The pattern of death in rats or rabbits after intravenous injection of the haemolytic fraction was essentially similar. Death in rats occurred after the administration of doses ranging from 2 to 9 M.U., which were injected into the tail vein over a standard 10 s period. The time to death varied from 5 to 45 min (nine rats). Rabbits died 5–12 min after doses ranging from 10–15 M.U. were injected into the marginal ear vein (four rabbits).

Rats died 5–40 min after doses of the cardiotoxic fraction ranging from 0·3 to 2·5 M.U. (nine rats). The results were qualitatively different in the two species, in that symptoms persisted in the rat for 30–40 min after near lethal doses, whereas under the same circumstances rabbits showed complete recovery after 5–8 minutes. The lethal dose for the rabbit ranged from 8–12 M.U., and death occurred after 3–5 min (four rabbits).

Cardiovascular effects

The cardiovascular changes brought about by either toxin were sufficiently similar for them to be described together. Initially there was a rapid rise in arterial pressure, which increased by as much as 50 mmHg (1 mmHg$\equiv$1·333 mbar) over a period of 15–30 seconds. This was followed by a fall in blood pressure, which was frequently precipitous. This hypotensive episode was always associated with a period of apnoea which varied in duration from 10 to 120 seconds. During this period of apnoea the arterial pressure rose again, usually to above the control level. There was then a second fall in arterial pressure, and some animals ceased breathing during this second hypotensive period and did not recover. In others a series of violent arterial pressure oscillations preceded death. These effects are illustrated in Figs. 2 & 3.

During the arterial pressure oscillations the electrocardiogram showed abnormal patterns. Bradycardia was seen within one minute of the injection of either toxin. There was then some increase in T wave amplitude, which was followed by depression of the S-T segment. Thereafter the traces showed intermittent atrioventricular blockade, and occasionally periods of ventricular tachycardia. The irregularities increased as death approached, and were associated with variable rises in right atrial pressure. A few animals showed terminal pulmonary oedema and blood stained fluid collected in the tracheal cannula.

Effects on carotid occlusion reflexes

The question arises as to whether the arterial pressure oscillations, which are a constant feature of death, are due simply to fluctuations in cardiac function against

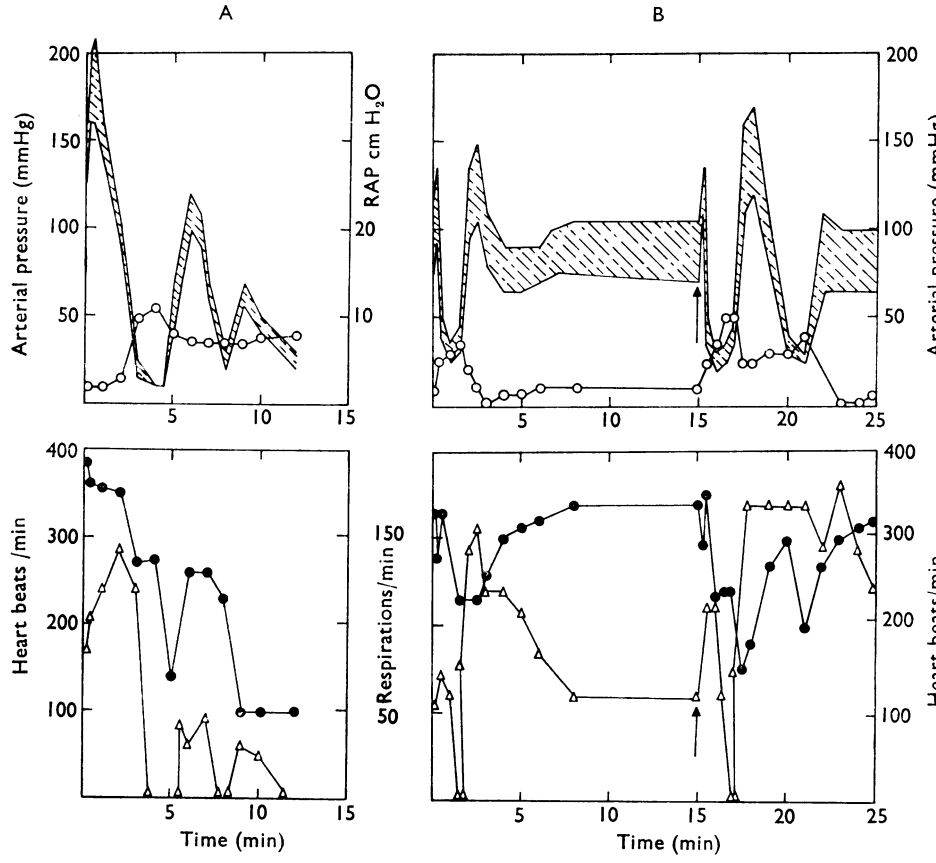

FIG. 2. The effect of the cardiotoxic fraction on systolic and diastolic pressure (hatched area), right atrial pressure (○——○), heart rate (●——●), and respiratory rate (△——△) in rat (A) and rabbit (B). The rat received 1 M.U. at time zero, the rabbit received 3 M.U. at time zero and 6 M.U. at 15 minutes. The rabbit recovered.

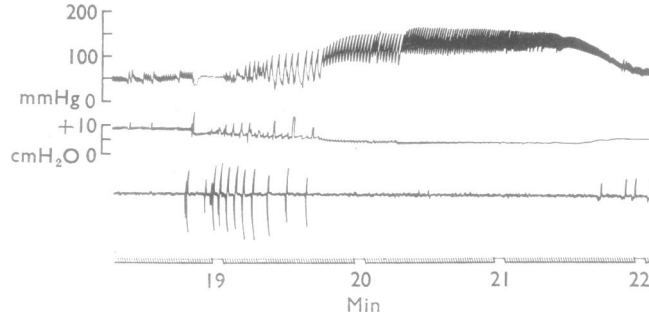

FIG. 3. The effect of 8 M.U. of haemolytic fraction given at zero time on arterial pressure (upper trace), right atrial pressure (middle trace), and respirations (lower trace). The rat was moribund at 19 min; after a period of gasping respirations heart rate and arterial pressure rose and atrial pressure fell, although the animal was apnoeic for 2 minutes. The atrial cannula appeared to block at 21 minutes.

a constant background of vasoconstriction or to some more complex mechanism. The oscillations suggest a disordered feed-back system, and raise the possibility that the toxin has brought about a disturbance of vasomotor reflexes. Consequently, experiments were designed to test the possibility that *Chironex* toxins interfere with some part of the vasomotor reflex pathway.

Carotid occlusion reflexes were elicited by clamping the carotid artery of the rat for 30 s, or by clamping both carotid arteries of the rabbit, in which arterial cannulation had been performed on the femoral artery. The resulting pressure increase averaged 30 mmHg. The effects of the toxins on this reflex were determined after low doses, so that periods of relatively stable hypotension lasting 4–5 min were available after the initial hypertensive response. Attempts were made to elicit the reflex when the arterial pressure in the rat varied from 47–70 mmHg, respirations varied from 25–65/min and the heart rate from 260–380/minute. It was not possible after either toxin to elicit a pressor response to carotid occlusion. However, (−)-noradrenaline bitartrate (1–2 μg/kg) given soon after such attempts elevated the arterial pressure by approximately 70 mmHg. It was noted, however, that noradrenaline given close to death was ineffective, possibly because of the generalized circulatory failure.

In the rabbit the carotid occlusion reflex was negative for from 6 to 10 min after a non-lethal dose of either toxin. Because of the short duration of symptoms after the cardiotoxin, the arterial pressure had returned to a near normal level before carotid occlusion was performed. The brevity of action of the both toxins in the rabbit led to a shorter duration of depression of the carotid occlusion reflex in this animal than in the rat.

Because of the sensitivity of carotid occlusion reflexes to anoxaemia a series of experiments was carried out in which rabbits were ventilated with a Palmer pump throughout the period of action of the toxins. Spontaneous respirations were suppressed with (+)-tubocurarine, 0·2 mg/kg given as required. Following non-lethal doses of the cardiotoxic fraction the arterial pressure changes were similar to those seen in the non-ventilated rabbit save that the second rise in arterial pressure was not seen unless the animal suffered a prolonged period (nearly 2 min) of acute hypotension. This was presumably responsible for a stagnant hypoxia sufficient to excite chemoreceptors, despite the maintenance of pulmonary ventilation. Carotid occlusion reflexes could not be elicited for periods of 4–8 min after injection of the cardiotoxin, depending on dose. The systolic pressure was 90–120 mmHg when the reflex was absent. Further, suppression of the reflex was not dependent upon a prior episode of hypotension, which might have depressed the vasomotor centre.

Effects of vagotomy

Bilateral cervical vagotomy was carried out in both rabbits and rats. It had but little effect on the development of symptoms, as was noted for the crude toxin preparation (Freeman & Turner, 1969). Respirations were slowed by acute section of the vagi, and in no instance was there a significant respiratory acceleration after injection of either toxin. Arterial pressure oscillations developed in the usual way. However, the clear cut relationship between apnoea and the pressor episodes was lost, so that apnoea sometimes preceded the arterial pressure increase, which was coincident with the return of respiratory activity.

Carotid sinus denervation

Section of the superior laryngeal, the recurrent laryngeal, the aortic depressor nerves, the cervical vagi and sympathetic trunks was carried out in the rat, and the nerves arising in the carotid bifurcation were also cut in the contralateral carotid to the arterial cannulation.

The arterial pressure in such rats always exceeded 200 mmHg. It was found, however, to be somewhat unstable, and even such a mild cardiac insult as inserting the right atrial cannula could precipitate a prolonged period of hypotension. One rat died in this way, although at autopsy there was no macroscopically evident damage.

Another rat suffered a precipitous fall in arterial pressure following insertion of the atrial cannula. In 40 s following atrial cannulation the arterial pressure fell from 195 mmHg to 30 mmHg. At this point the animal stopped breathing for 90 seconds. Coincident with the return of respiratory activity the pressure rose again, and a series of slow pressure oscillations preceded death some 40 min later. In each instance the pressor response was preceded by a period of apnoea. It would seem that without the buffering action of peripheral baro- and chemoreceptors pressure oscillations can occur which resemble approximately those induced by the toxins. The periodicity of the oscillations was too slow for them to be 'Mayer waves' (Heymans & Neil, 1958); however, it will be recalled that the sinus nerve of the cannulated carotid was not cut and an increase in flow in the pharyngeal-carotid anastomoses during pressor episodes could have reduced carotid chemoreceptor drive, leading to oscillations. Because of the hazards of atrial cannulation this was abandoned for this series of experiments.

Although the rats were hypertensive due to baroreceptor denervation the injection of either toxin still brought about a further increase in arterial pressure by 10–30 mmHg. This was followed by a slower than usual fall in pressure and a period of apnoea, which was succeeded by one or two pressure oscillations. As can be seen from Fig. 4 denervation reduced the respiratory rate from the control value of 74/min (Table 1) to 54 ± 5 (S.E.M., eight rats). The heart rate was not significantly changed at 367 ± 18/minute.

However, cardiac and respiratory difficulties developed in the usual way after the injection of either toxin, save for the absence of respiratory stimulation before apnoea. It was noteworthy, though, that unlike rats with the baroreceptor innervation intact, the arterial pressure increase did not occur until respirations had resumed. This was a constant finding with both toxins.

Hexamethonium

If the arterial pressure oscillations are ascribed to factors additional to fluctuations in cardiac function, then interference with any part of the vasomotor reflex pathway should reduce or abolish such oscillations. Hexamethonium was injected into rabbits and rats in divided doses of 2·5 mg/kg, until the arterial pressure had fallen to a steady level and it was no longer possible to elicit a carotid occlusion reflex.

Usually a total dose of 10 mg/kg was given intravenously over 20 minutes. The arterial pressure in the rat was reduced to 91 ± 6 mmHg (S.E.M., nine rats). Injection

of either toxin after ganglionic blockade brought about the usual pressor response, which averaged 60 mmHg above the base line level. Thereafter the arterial pressure fell; pressure oscillations were either entirely absent or were slow, small in magnitude (20–30 mmHg), and unrelated to periods of apnoea. Respiratory and electrocardiographic changes followed the usual pattern, save that at no time was the respiratory rate increased above the control level. Closely similar results were obtained with rabbits.

These results confirm the previous findings (Freeman & Turner, 1969) and suggest that the initial pressor response can be ascribed to a direct vasoconstrictor effect of

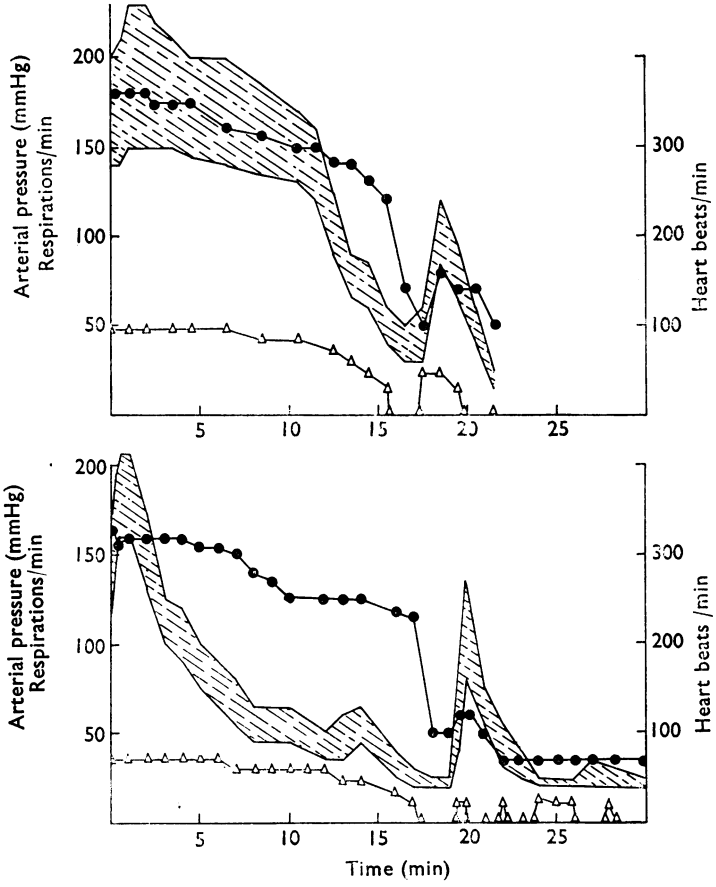

FIG. 4. Upper diagram: the effect of the haemolytic fraction (5 M.U.) in the rat after radical section of the sinoaortic afferents; lower diagram: the effect of the cardiotoxic fraction (2·5 M.U.) in the rat after section of the same nerves. The animal died at 30 minutes. Systolic and diastolic pressure (hatched area), heart rate (●——●), and respiratory rate (△——△).

TABLE 1. *Control values for physiological parameters in rabbits and rats*

	Systolic pressure mmHg	Diastolic pressure mmHg	Right atrial pressure cm H_2O	Respiratory rate/min	Heart rate/min
Rat	161±4 (33)	127±3 (33)	0·2±0·4 (33)	74±3 (33)	372±10 (33)
Rabbit	125±4 (10)	91±4 (10)	−0·2±0·7 (10)	39±3 (10)	300±8 (10)

Figures shown are ±S.E.M. The number of observations is shown in parentheses.

either toxin. It is possible, however, that the effect could be mediated by catecholamine release, in a manner similar to that of indirectly acting sympathomimetic amines.

Phenoxybenzamine

The possibility of catecholamine release was tested in rats and rabbits pretreated with phenoxybenzamine hydrochloride (2–4 mg/kg). The animals received the α-adrenoceptor blocking agent intravenously 2 h before the administration of either toxin. These doses completely inhibited the carotid occlusion reflex and the response to noradrenaline. The arterial pressure was reduced to 117 ± 7 mmHg (S.E.M., six rats) and 70–80 mmHg (four rabbits). Right atrial pressure, heart rate and respiratory rate were not significantly altered in either species.

The initial pressor response to either toxin was somewhat enhanced in both species. It was also sustained for longer after both toxins in the rat. At no time were pressure oscillations seen. The picture differed somewhat in the rabbit, where the initial pressor response was followed by a rapid fall in arterial pressure after both toxins. There was then a period of apnoea of 20–30 s duration. The arterial pressure rose again to slightly higher than the base line level, concurrent with the resumption of respirations. It then slowly returned to normal. The hypotensive episode was always associated with a sharp but transitory rise in right atrial pressure, and it can be assumed that the hypotension was due to a temporary fall in cardiac output. After lethal doses of either toxin the rabbit died during this first hypotensive period, without showing any further pressure oscillations.

It was noteworthy that the lethal dose of the haemolytic fraction, in the phenoxybenzamine treated rabbit, was raised from 10–15 M.U. to 50–60 M.U. There was a similar but less clear cut tendency in the rat.

Bretylium

Intravenous injection of bretylium tosylate (10 mg/kg) into rats about 20–30 min before toxin injection did not affect the initial pressor response to either toxin. Hypotension and an increase in right atrial pressure followed this response. The rate of decline of arterial pressure was dose dependent, and there were no pressure oscillations associated with the apnoeic periods.

Propranolol

Intravenous injection of 0·1 mg/kg propranolol did not affect the response of the rat to either toxin. However, 0·4 mg/kg propranolol markedly damped the arterial pressure oscillations. This may reflect the local anaesthetic action of this concentration of β-adrenoceptor blocking agent (Shanks, 1967).

Effects on the isolated, perfused guinea-pig heart

It was shown previously (Turner & Freeman, 1969) that low doses of the crude toxin preparation brought about reversible decreases in rate, amplitude of contraction and coronary flow in the isolated guinea-pig heart perfused under constant pressure. The present experiments were carried out to determine whether the two toxins are of equivalent potency as cardiotoxins.

TABLE 2. *Effect of the cardiotoxic and haemolytic fractions on the isolated heart*

	Heart rate percentage of control at 1 min			Coronary flow percentage of control at 30 s			Amplitude of contraction percentage of control at 1 min		
M.U.	0·03	0·3	1·0	0·03	0·3	1·0	0·03	0·3	1·0
Cardiotoxin	97·7±1·0 (5)	93·8±1·0 (5)	93·0±0·9 (4)	55·0±4·4 (5)	36·6±3·2 (5)	34·1±6·0 (4)	60·6±12·6 (5)	37·3±15·0 (5)	46·5±16·8 (4)
Haemolysin	96·1±0·7 (6)	91·2±2·1 (3)	92·4±1·5 (6)	95·8±3·7 (6)	76·3±5·7 (3)	54·6±5·8 (6)	96·7±4·0 (6)	76·8±5·8 (6)	56·5±15·4 (6)

Figures shown are ±S.E.M. The number of observations is shown in parentheses.

Toxin samples were injected into the perfusion system just proximal to the heart. The perfusate was not recycled, therefore the heart was exposed only once to each toxin. Control values for heart rate, coronary flow and amplitude of contraction were within the range determined previously (Turner & Freeman, 1969). Injections of either toxin ranging from 0·03 M.U. to 1·0 M.U. reduced the heart rate by less than 10 per cent. Coronary flow was also reduced by both toxins. However, 0·03 M.U. of the cardiotoxic fraction reversibly reduced the flow rate to an average of 55 per cent, whereas 1·0 M.U. of the haemolytic fraction was needed to bring about a comparable reduction in flow. Similarly, low doses of cardiotoxin brought about a greater reduction in amplitude of contraction. The duration of action of the toxins also differed. The cardiotoxic fraction brought about a marked but short-lived reduction in cardiac function, whereas the effects seen after the haemolytic fraction were slower in onset, and at the highest concentration were longer in duration. These effects are consistent with those seen in the intact animal. The variability of the data can be assessed from Table 2, which shows the percentage changes in coronary flow at 30 s, and in amplitude and rate at 1 minute. Thus low doses of the cardiotoxic fraction are a more effective cardiac depressant than are equivalent doses of the haemolytic fraction, although both can bring about ventricular asystole in larger doses.

Further experiments were conducted in which the heart was perfused under constant flow conditions at 10 ml/minute. Doses of 0·03 or 0·3 M.U. of the cardiotoxic fraction increased coronary perfusion pressure to 160–170 per cent of the control value (48 mmHg). The increase was short-lived and perfusion pressure returned to normal within 4–6 minutes. Decreases in heart rate were similar to those observed previously. The amplitude of contraction was also decreased, although the decrease amounted to only 50 per cent of that observed in the constant pressure experiments. The shorter time of exposure to the toxin under constant flow conditions may account for this difference.

Discussion

The two toxins that have been isolated from *Chironex fleckeri* have a number of properties in common. They are both unstable, both are cardiotoxic, but only the lower molecular weight toxin is haemolytic. Both cause vasoconstriction apparently by a direct action on vascular smooth muscle. The brief duration of this action on the coronary bed suggests that the effect on the systemic circulation may also be transient.

The evidence put forward in the present study suggests that the cardiovascular picture produced by cardiotoxicity and vasoconstriction may be complicated by baroreceptor stimulation and possibly a depressant action on the brain stem vasomotor centre. The profound blood pressure oscillations seen after both toxins suggest a disordered feed-back system such as could occur after interference with vasomotor reflexes. Thus the initial arterial pressure rise will be due to constriction of the vascular musculature; subsequent hypotension can be ascribed to a combination of baroreceptor stimulation which will reduce vasomotor tone, and a fall in cardiac output. The subsequent apnoea can be reflexly induced by baroreceptor stimulation, and will lead to excitation of peripheral and central chemoreceptors, and thus increase the efferent vasomotor outflow. The arterial pressure

will rise until restitution of respirations reduces the chemoreceptor drive, when the arterial pressure will fall again. Thus blood pressure oscillations could continue until either the heart or the CNS ceases to function effectively.

Evidence for the involvement of some part of the baroreceptor reflex arc is given by the depression of the carotid occlusion reflex at a time when vasoconstriction by noradrenaline was demonstrable. This depression was seen in both species after either toxin. The finding is particularly relevant in the rabbit, where the reflex was absent at a time when the arterial pressure was nearly normal. Thus the reflex cannot have been inactivated by hypotension *per se*.

The occurrence of oscillations in blood pressure after baro- and chemoreceptor denervation is consistent with this hypothesis since the blood pressure fall after the initial pressure response was slower than usual, and the oscillations were in phase with the periods of apnoea, rather than out of phase as was seen in the fully innervated animal. Peripheral chemoreceptor stimulation due to hypercapnia appears to raise the blood pressure sooner than stimulation of the respiratory centre leads to the restitution of respirations. When these chemoreceptors are denervated the blood pressure rise and the return of respirations are synchronized. However, these experiments do not conclusively differentiate between baroreceptor stimulation and depression of the vasomotor centre. Hypoxia and inadequate perfusion must inevitably interfere with CNS function. The large size of the toxin molecules suggests that they will penetrate the brain capillaries slowly if at all, and favours the concept of a peripheral action.

Interference with ganglionic transmission (hexamethonium), adrenergic nerve terminal transmission (bretylium) or the α-adrenoceptor (phenoxybenzamine), all either greatly diminish or abolish the blood pressure oscillations. If the efferent arm of the reflex arc is blocked the opposing effects of baro- and chemoreceptor stimulation will be ineffective and the cardiovascular picture will result simply from transient vasoconstriction and cardiac depression.

The clear-cut relationship between the arterial pressure oscillations and periods of apnoea which was seen after the isolated toxins was not seen when a crude toxin preparation was used (Freeman & Turner, 1969). The slightly different rate of onset of effects of the two toxins suggests that their combined injection during envenomation will cause symptoms due to interactions between these effects.

It is not possible to assess the relative importance of these factors in determining the clinical picture in man. However, the great development of vasomotor reflexes in man, because of his erect posture, could indicate that baroreceptor stimulation plays an important part in the action of the toxins. Barnes (personal communication) has noted that victims of *Chironex* stings frequently ' rally ' just before death. This may correspond to the situation in the experimental animal. The study is continuing.

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