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SOME EFFECTS OF STAPHYLOCOCCAL α-TOXIN ON THE CARDIOVASCULAR SYSTEM

BY

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Staphylococcal α-toxin has been found to release histamine from perfused cat lungs (Feldberg and Keogh, 1937) and from isolated cat skin (Brown, 1965). However, as the latter worker observed that intravenous injections of lethal doses of α-toxin into anaesthetized cats did not increase noticeably the concentration of histamine in the blood plasma, histamine-release *in vivo* seemed unlikely to be the major factor responsible for the action of the α-toxin in the whole animal. For this reason it was necessary to assess the role of the other actions of the α-toxin in an attempt to discover which predominated in the lethal action of the toxin.

A classical investigation into the effects of staphylococcal culture filtrates on the circulation of the cat was made by Kellaway, Burnet & Williams (1930) who found that the filtrate produced a triphasic blood pressure change when injected intravenously—an initial and immediate fall, then a rise or recovery to near normal values and finally a prolonged fall of pressure ending in death. They considered that, as vasodilatation occurred during the initial depression but not during the final fall, death resulted from cardiac failure for which there were two possible causes: first, pulmonary obstruction (shown by a rise in pulmonary arterial pressure and post-mortem distension of the right heart and pulmonary arteries) and second, coronary vasoconstriction seen in isolated hearts perfused via the coronary arteries. The latter effect has also been reported by Wiegershausen (1962).

In the present study, the effects of a preparation of the staphylococcal α-toxin used by Brown, Prichard & Quilliam (1959) on the blood pressure and peripheral blood flow of the anaesthetized cat and on isolated coronary-perfused cat and rabbit hearts have been re-examined. My results with α-toxin accord with those of Kellaway *et al.* (1930) using staphylococcal filtrates and show that the α-toxin has a strong peripheral vasoconstrictor action.

METHODS

Cats were anaesthetized with sodium pentobarbitone (35 to 45 mg/kg intraperitoneally) or with chloralose (60 to 80 mg/kg intravenously) after induction with diethylether. Mean arterial blood pressure was recorded from the carotid or femoral arteries using a mercury manometer. In some experiments the venous outflow from the ear *in situ* (the preganglionic sympathetic nerve supply to which was sectioned acutely) was also measured using a photoelectric drop counter coupled to a Thorpe impulse counter. The effects of changes of the arterial blood pressure on the blood

flow through the ear could be excluded by connecting the blood pressure manometer to a 25 l. reservoir of air and a bottle containing 6% dextran in 0.9% (w/v) saline solution. A fall of blood pressure was thereby countered by the infusion of dextran solution into the circulation and a rise by the effusion of blood. Drugs were injected intravenously via the femoral vein.

Isolated cat or rabbit hearts were perfused through the aorta and coronary vessels (the Langendorff perfusion technique) with filtered Krebs solution at 37°, equilibrated with a 95% oxygen/5% carbon dioxide gas mixture. The amplitude of the ventricular contraction was recorded on a smoked paper using a Starling spring-loaded heart lever. The ventricular rate was recorded by allowing the lever movement to trigger a Thorpe impulse counter. The coronary flow was measured by directing the outflow of perfusate from the heart into a volume recorder based on that described by Bülbring, Crema & Saxby (1958). This in turn was connected to a float recorder writing on the smoked drum, so that each 2 ml. of outflow induced a single excursion of the float. Drugs were injected into the ingoing perfusion stream, just above its point of entry into the aorta.

Drugs Wellcome staphylococcal α-haemolysen (α-toxin) and anti-α-haemolysen (anti-α-toxin) were used (see Brown *et al.*, 1959). Doses are given in haemolytic units (H.U.) and international units (I.U.) respectively.

Doses of histamine acid phosphate, 5-hydroxytryptamine creatinine phosphate, adrenaline acid tartrate, isoprenaline sulphate, mepyramine maleate, tolazoline hydrochloride, methysergide hydrogen maleate (Deseril, Sandoz), and sodium nitrite refer to the weight of the salt. Doses of heparin, compound 48/80, aminophylline, papaverine and amyl nitrite are expressed as the weight of the pure compound.

RESULTS

Effects on blood pressure and peripheral blood flow

Effects of intravenous injections of 0.5 to 10 H.U./kg of staphylococcal α-toxin on the mean arterial blood pressure were recorded in 13 cats: of these, five were bilaterally vagotomized and artificially ventilated (one had also been adrenalectomized bilaterally), two had been pretreated with a combination of atropine (1 mg/kg), hexamethonium (1 mg/kg) and tolazoline (10 mg/kg) and one was pretreated with 300 I.U./kg of staphylococcal anti-α-toxin.

In all cats except for the one pretreated with antitoxin, a sharp fall of blood pressure occurred immediately after the injection of α-toxin. The subsequent sequence of events varied. In two out of 12 cats the initial fall was sustained and led rapidly to the death of the animal. In the remaining 10 cats partial recovery to the initial blood pressure value or an overt pressor response (Fig. 1) intervened before the terminal fall of blood pressure. Death occurred 10 to 30 min after the injection of 5 or 10 H.U./kg of α-toxin. Lower doses did not lead to death. In three cats the terminal fall of blood pressure was preceded by, or accompanied by, irregularities in the blood pressure trace suggestive of cardiac arrhythmias (Fig. 1). The effect of the toxin was unaffected by the pretreatments of the animal, except that after the antitoxin doses of up to 30 H.U./kg of α-toxin were without effect on the blood pressure.

In four of the above cats (all under artificial ventilation and in one also with bilateral adrenalectomy) the venous outflow from the ear was recorded simultaneously with the blood pressure (Fig. 2). Intravenous injection of α-toxin produced an immediate, transient increase in blood flow, coinciding with the initial fall of blood pressure. This was followed by a severe reduction of outflow which lasted until death. The reduced outflow was due to peripheral vasoconstriction because it also occurred in three further experiments in which the blood pressure was stabilized. Adrenalectomy did not appear to modify

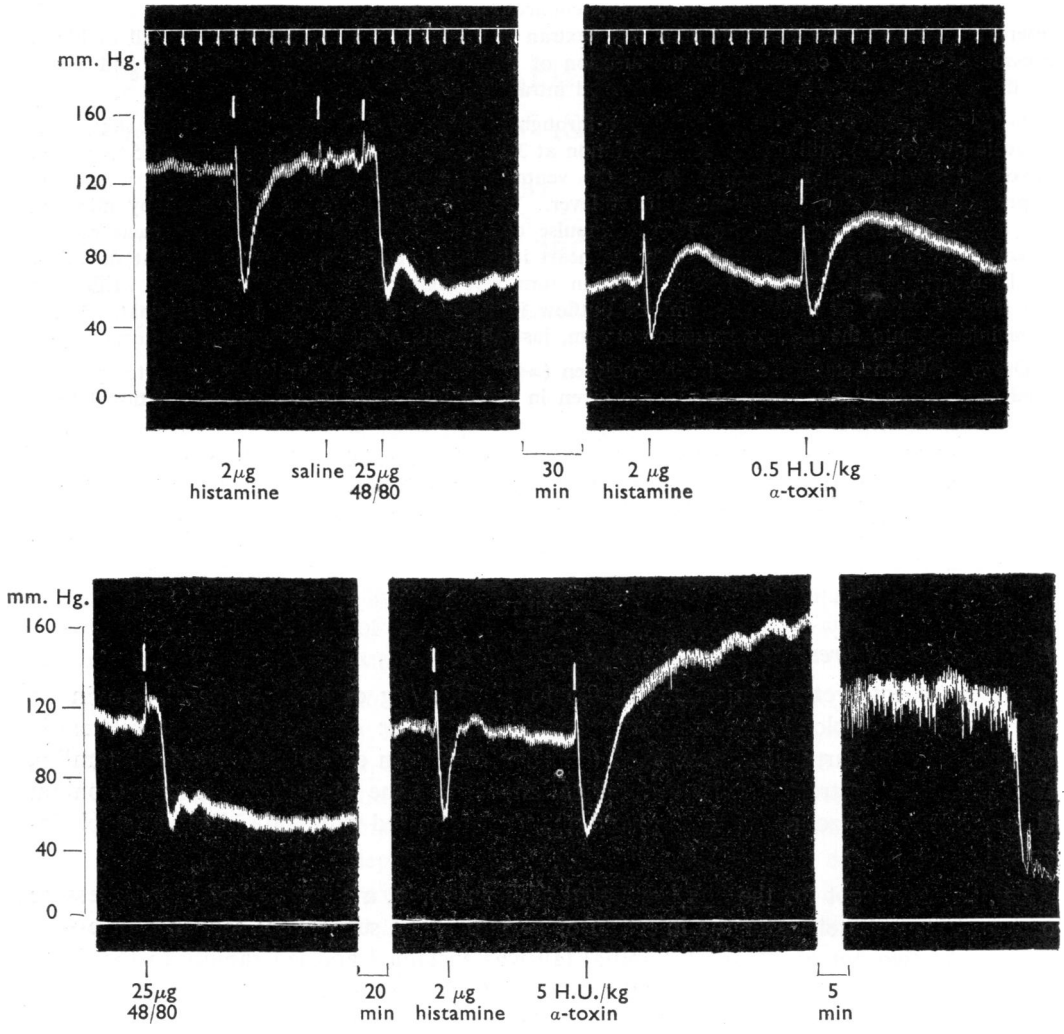

Fig. 1. Comparative effects of histamine, compound 48/80 and staphylococcal α-toxin on the carotid arterial blood pressure of a cat anaesthetized with pentobarbitone. The upper and lower records were obtained in the same experiment, with an interval of 20 min between them. Other intervals are indicated on the trace. Drugs were injected intravenously via the femoral vein, dissolved in 0.5 ml. of normal saline. The last dose of α-toxin was lethal, death being heralded by the abrupt fall of blood pressure in the last trace. Note the appearance of cardiac irregularities prior to the terminal fall of blood pressure. Time, 30 sec.

the vasoconstrictor effect. Inhalation of amyl nitrite vapour or intravenous injection of papaverine (1 mg) restored transiently the blood flow but did not prevent death. Intravenous injections of histamine (5 to 20 μg) produced an immediate and sharp increase and compound 48/80 (25 to 50 μg) a delayed increase in blood flow.

Toxin-induced death was neither accompanied by overt signs of respiratory distress nor was it delayed in cats receiving artificial ventilation. Prior to death the pupil was

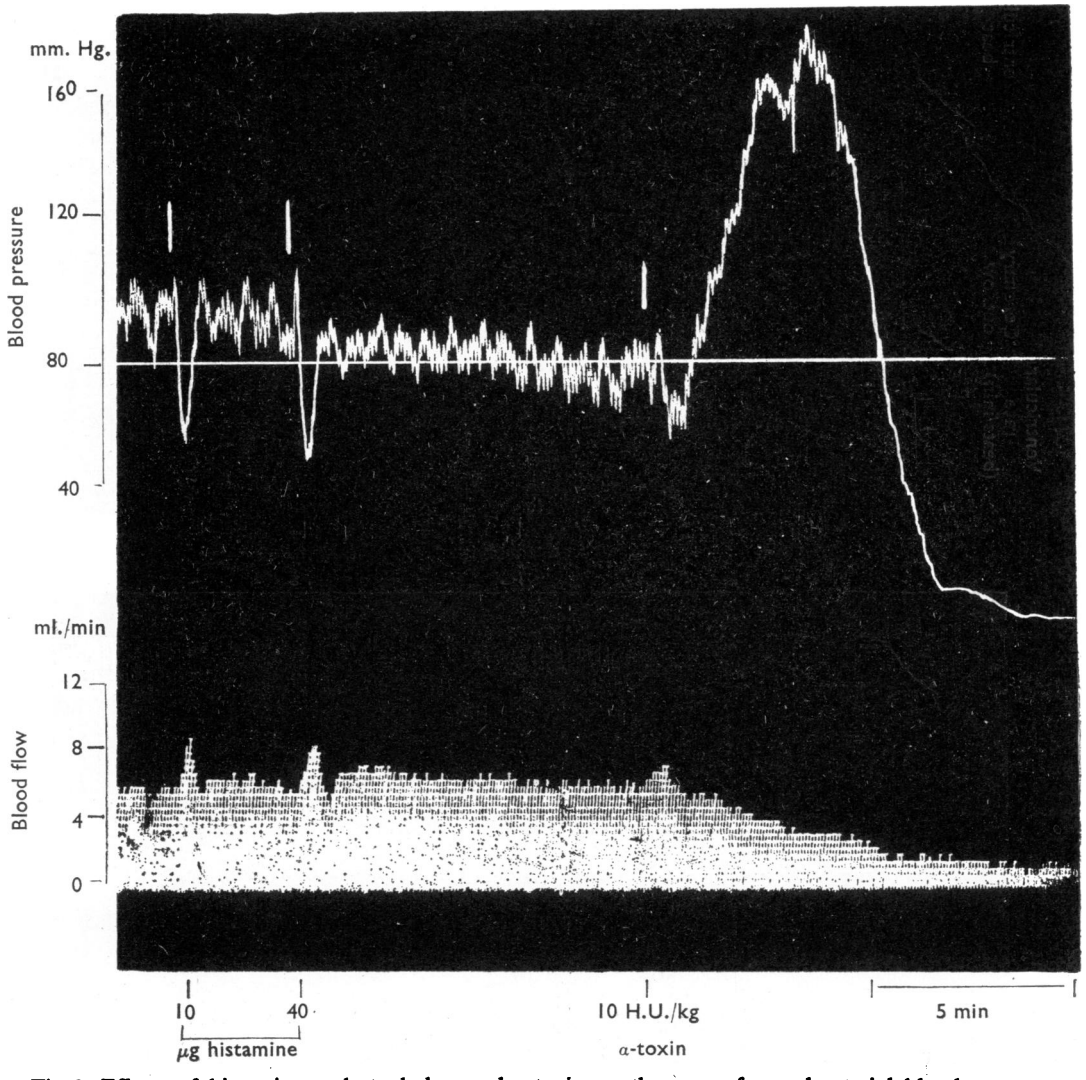

Fig. 2. Effects of histamine and staphylococcal α-toxin on the mean femoral arterial blood pressure (upper trace) and the venous outflow from the ear (lower trace) of a cat anaesthetized with chloralose and receiving artificial ventilation after bilateral vagotomy. The final fall of blood pressure was associated with the death of the cat.

constricted, but the nictitating membrane was retracted. Post-mortem, the heart and large arteries, particularly the right atrium and ventricle and pulmonary arteries, were distended with blood. The intestines were pale and devoid of blood. No haemolysis was detected, except after 100 H.U./kg of α-toxin (see Brown, 1965).

Effects on the isolated heart

Coronary outflow and the amplitude and rate of the ventricular contraction were recorded in five isolated cat hearts and 17 isolated rabbit hearts perfused by the

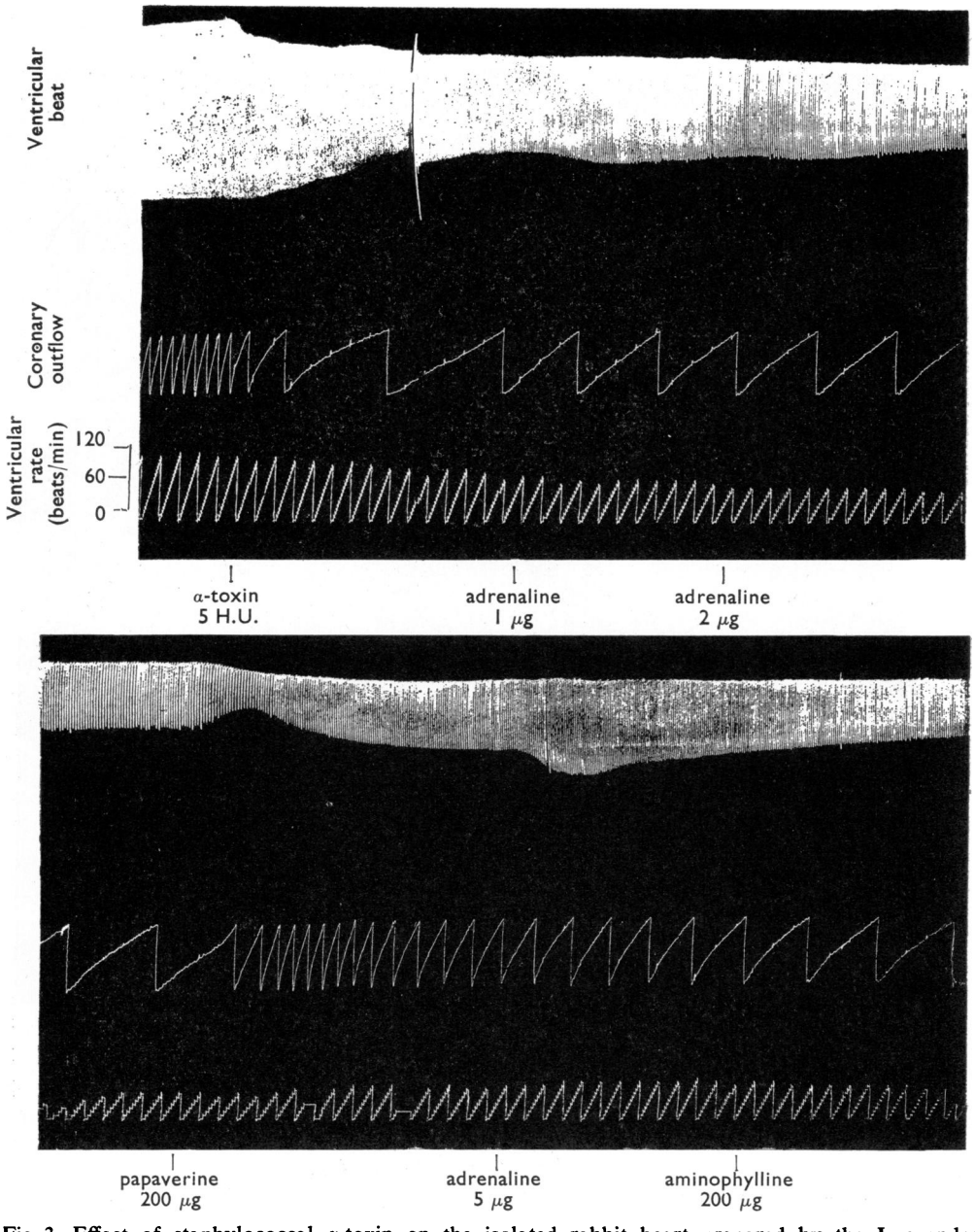

Fig. 3. Effect of staphylococcal α-toxin on the isolated rabbit heart prepared by the Langendorff method. The lower trace is a continuation of the upper, separated by an interval of 1 min. Records, from above downwards, show: amplitude of ventricular contraction (downward lever movement); coronary outflow, each vertical upward excursion of the recorder indicating 2 ml. of outflow; and ventricular rate, with the rate recorder reset at 10 sec intervals. The perfusion fluid was filtered Krebs solution at 37°, equilibrated with 95% oxygen/5% carbon dioxide mixture. Drugs, dissolved in 0.5 ml. of normal warmed saline solution, were injected into the perfusion stream just above its point of entry into the aorta. Papaverine reversed the coronary constrictor action of the α-toxin. Aminophylline was ineffective in a dose of 200 μg, but larger doses were effective (see Table 2 and Fig. 4).

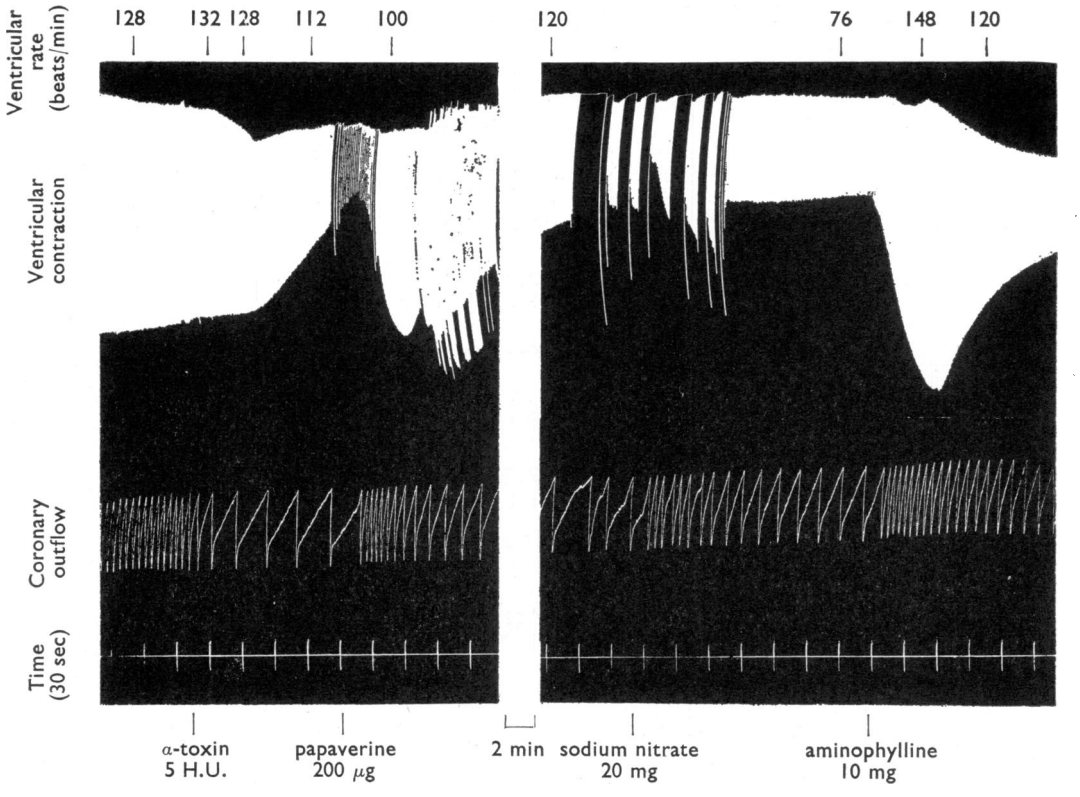

Fig. 4. Action of staphylococcal α-toxin on the isolated Langendorff-perfused cat heart. Records, from above downwards, show: amplitude of ventricular contraction; coronary outflow (calibrated as in Fig. 3); and time, in 30 sec steps. The rate of the ventricular contraction, counted over 15 sec periods but expressed as beats/min, is indicated over the trace. Conditions of perfusion and drug injection as in Fig. 3. Note that the drum speed was half that in Fig. 3.

Langendorff method and the effects of injections into the perfusion fluid of 0.1 to 20 H.U. of staphylococcal α-toxin on these parameters of cardiac function were studied (Figs. 3 & 4; Table 1).

The most striking change after the injection of α-toxin was a large reduction of coronary outflow. Expressed as % of the resting outflow, the reduction of outflow varied from 30% after the injection of 0.1 H.U. of α-toxin to about 90% after 20 H.U. of α-toxin in the isolated rabbit heart (Table 1). Similar reductions of outflow were seen with isolated cat hearts, including one preparation exhibiting spontaneous ventricular fibrillation. It was not possible to determine precisely the time after the injection of α-toxin at which the outflow became impaired, because there was a lag in the response of the recorder which varied with the initial rate of flow. However, it appeared that the flow was reduced within a few seconds of injection.

Injection of α-toxin also led to a slow and progressive decline in the amplitude and rate of the ventricular beat. These inotropic and chronotropic effects occurred in both

TABLE 1

EFFECT OF STAPHYLOCOCCAL TOXIN ON CORONARY OUTFLOW, AMPLITUDE OF VENTRICULAR CONTRACTION, AND VENTRICULAR RATE IN THE ISOLATED RABBIT AND CAT HEART

			% Reduction (−) or increase (+) of:											
			Coronary outflow				Amplitude of ventricular contraction				Ventricular rate			
Species	Conditions	Dose of Toxin (H.U.)	Mean	Range	S.E.	n.	Mean	Range	S.E.	n.	Mean	Range	S.E.	n.
Rabbit (17 hearts)	Normal	0·1	−27	−20 – −33	—	2	− 7	− 2 – −12	—	2	− 7	0 – −14	—	2
	Normal	0·5	−59	−57 – −61	—	2	−23	−21 – −25	—	2	−10	0 – −19	—	2
	Normal	5	−78	−55 – −88	4·6	8	−35	− 2 – −80	10·3	8	−11	−8 – −17	2	4
	Normal	20	−89	−88 – −90	—	2	−44	−40 – −48	—	2	−33	—	—	1
	+ Antitoxin 5 I.U./ml.	5	− 2	0 – − 4	—	2	+ 2	− 1 – + 4	—	2	− 7	—	—	1
		20	−44	−20 – −68	—	2	−21	−10 – −32	—	2	− 4	—	—	1
	+ {Methysergide 5×10^{-8} / Mepyramine 2×10^{-7}	5	−82	−74 – −88	3·2	4	−20	0 – −56	10·3	4	− 3	+ 2 – − 8	3	4
	+ Papaverine 5×10^{-8}	5	−16	—	—	1	+12	—	—	1	+20	—	—	1
Cat (5 hearts)	Normal	1	−63	—	—	1	+ 1	—	—	1	−31	—	—	1
	Normal	5	−82	—	—	1	−25	—	—	1	−30	—	—	1
	+ {Methysergide 8×10^{-8} / Mepyramine 2×10^{-7}	5	−83	−81 – −85	—	2	−40	−21 – −60	—	2	−18	−15 – −22	—	2
	With ventricular fibrillation	1	−18	—	—	{1	—				—			
		5	−62	—	—		—				—			

TABLE 2

EFFECTS OF SOME CORONARY DILATOR DRUGS IN REVERSING THE DIMINUTION OF CORONARY OUTFLOW FROM THE ISOLATED RABBIT HEART PRODUCED BY STAPHYLOCOCCAL α-TOXIN

Full restoration of coronary outflow to pre-toxin level is indicated by +100%. Further depression of coronary outflow in excess of that produced by α-toxin is indicated by negative value: abolition of flow = −100%

Drug	No. of experiments	Dose	% Restoration (+) or depression (−) of coronary outflow		
			Mean	Range Min.	Range Max.
Papaverine	11	50–200 μg.	+45	+13	+108
Sodium nitrite	5	20–50 mg.	+43	+16	+ 72
Aminophylline	4	0·2–0·5 mg.	+ 4	− 3	+ 16
Aminophylline	4	10 mg.	+24	+14	+ 30
Isoprenaline	2	0·2–0·25 μg.	+ 6	+ 6	+ 7
Adrenaline	7	0·2–2 μg.	+ 5	− 8	+ 37

species after the change of outflow began (see Figs. 3 & 4). Negative inotropic and chronotropic effects, measured at the time of maximum effect on coronary outflow, were rather smaller and more variable than the changes of coronary outflow. (Table 1.)

Addition of 5 I.U./ml. of staphylococcal anti-α-toxin to the perfusing fluid protected the heart completely from the effects of 5 H.U. of α-toxin and considerably reduced the effect of 20 H.U. of α-toxin. Addition of 50 μg/ml. of papaverine to the perfusing fluid also largely reduced the immediate effect of the α-toxin on the coronary outflow, and abolished its effect on ventricular contraction. However, when perfusion with normal Krebs solution was recommenced, a progressive diminution of coronary outflow and ventricular contraction occurred. This was not seen after substituting normal Krebs' solution for antitoxin solution.

The impairment of coronary outflow obtained after injection of α-toxin was transiently reversed by the subsequent injection of coronary vasodilator agents (papaverine, 50 to 200 μg ; sodium nitrite, 20 to 50 mg ; aminophylline, 10 mg). These agents were most effective when administered very shortly after the toxin, and had progressively less effect on repeated injection. They exerted very little or no effect when given 30 min or more after the initial toxin injection. Papaverine was the most effective agent tested in terms of both the dose required and the magnitude of the increase in flow (Table 2). The injection of papaverine initially depressed the ventricular contraction, but following the increased coronary outflow there frequently occurred a positive inotropic response (Figs. 3 & 4). This latter effect was more marked than that seen after the injection of papaverine into normal hearts. Aminophylline produced positive inotropic and chronotropic effects simultaneous with the increase in coronary outflow. Adrenaline and isoprenaline had very little effect on coronary outflow.

Wiegershausen (1962) has suggested that the reduction of outflow from the isolated heart produced by the α-toxin might be mediated through the local release of 5-hydroxytryptamine. Local release of histamine might also be considered from this point of

view. When tested on isolated cat and rabbit heart preparation, 5-hydroxytryptamine (20 and 100 μg) did not materially affect the coronary outflow, though eliciting positive inotropic and chronotropic effects. The action of 5-hydroxytryptamine on the heart was not affected by adding methysergide (0.05 μg/ml.) to the perfusion fluid. Histamine (20 μg) reduced the coronary outflow from the isolated rabbit heart by about half, but did not change the coronary flow in the isolated cat heart. Histamine exerted a marked positive inotropic and a slight positive chronotropic effect in both species. Mepyramine (0.2 μg/ml.) antagonized the effect of histamine on coronary outflow, but not the chronotropic and inotropic effects of histamine. A combination of mepyramine (0.2 μg/ml.) with methysergide (0.05 μg/ml.) in the perfusion fluid did not modify the response of the isolated heart to staphylococcal α-toxin.

DISCUSSION

The effects of staphylococcal α-toxin on the blood pressure and peripheral blood flow in the anaesthetized cat clearly cannot be ascribed in any large part to histamine release. Instead of the delayed depressor effect with peripheral vasodilatation characteristic of histamine liberators (MacIntosh & Paton, 1949), the α-toxin produced, after an initial fall of blood pressure, a pressor response with peripheral vasoconstriction (see Figs. 1 & 2). This accords with the observation of Brown (1965) that very large doses of α-toxin (100 H.U./kg) did not raise the plasma histamine concentration in cats.

The sequence of events following the injection of staphylococcal α-toxin in cats (initial fall of blood pressure, recovery or pressor response, then a terminal fall of pressure) reported in this paper accord well with the observations of Kellaway *et al.* (1930) with staphylococcal filtrates. The first depressor response is associated with peripheral vasodilatation, the recovery and second fall with marked peripheral vasoconstriction. The vasoconstriction is probably the result of a direct action of the α-toxin on the blood vessels. It could not have been mediated through afferent or central nervous effects because the sympathetic nerve supply to the vessels under observation had been sectioned. It is unlikely to have been induced by the discharge of adrenaline from the adrenal medulla because the same effect was seen in an adrenalectomized cat, and the pressor response was also observed in cats pretreated with tolazoline. Further evidence of a direct vasoconstrictor action of the α-toxin has been presented by other workers. Thal and Egner (1954) found that the direct application of α-toxin to transilluminated dog or rabbit mesentery produced a sustained spasm of arterioles and venules. Subsequently (in 1961), the same authors reported that the α-toxin produced vasoconstriction in the isolated perfused kidney, and also contracted isolated venous segments. Vasoconstriction in the isolated perfused rabbit ear preparation has been reported by Wiegershausen (1962).

The coronary vasoconstrictor action of the α-toxin, previously reported by Kellaway *et al.* (1930) and by Wiegershausen (1962), has been confirmed in the present study. Since this effect was seen on occasions in the absence of inotropic or chronotropic effects, and also in the fibrillating heart, it was unlikely to have been secondary to other cardiac effects. Indeed, the negative inotropic and chronotropic changes observed, particularly with the larger doses of α-toxin, were probably induced by the coronary constriction.

Firstly, the change of coronary flow invariably preceded other cardiac effects. Secondly, the effect of the α-toxin on coronary flow was more consistent and generally greater than its effect on ventricular function. Thirdly, papaverine temporarily prevented not only the coronary constrictor action of the α-toxin, but also other effects on ventricular contraction and rate. This view that the effect of the toxin on the heart beat results from diminished coronary flow is supported by observations that the α-toxin affects neither the spontaneous contractility of isolated cat auricles (Kellaway *et al.*, 1930), nor the response of isolated ventricular muscle strips to electrical stimulation (Thal and Egner, 1961).

There seems to be little supporting evidence for the view of Wiegershausen (1962) that the coronary vasconstriction arises from the local release of 5-hydroxytryptamine, since the effect of this agent differed from that of the α-toxin in several respects. For similar reasons, the coronary constriction is unlikely to have arisen from the release of histamine. The simplest explanation for the peripheral and coronary vasoconstrictor actions of the α-toxin is that of a direct action on vascular smooth muscle, similar to that on isolated intestinal and uterine smooth muscle (Brown *et al.*, 1959; Brown & Quilliam, 1965). The similarity between these actions is indicated by the finding that a strong vasodilator and spasmolytic agent such as papaverine can exert a temporary antagonism if administered soon after the α-toxin, but not if given some time after the contraction or vasoconstriction has developed. Also papaverine can prevent the development of either vasoconstriction or spasm of smooth muscle of the gut and uterus produced by the subsequent administration of α-toxin but for only as long as the papaverine is in contact with the tissue (see Brown *et al.*, 1959). Since both vasoconstrictor and spasmogenic actions of the α-toxin are notable for their persistence in the face of washing or of repeated administration of spasmolytic agents, this may indicate either persistent "binding" of the α-toxin to the smooth muscle cells, or perhaps irreversible "damage" to the cells. Histological evidence for damage to smooth muscle cells has been put forward by Thal and Egner (1961). It is of considerable interest that cardiac and skeletal muscle appears to be much more resistant to any action of the α-toxin than does smooth muscle (Thal and Egner, 1961; Brown, unpublished observations).

The observations of Kellaway *et al.* (1930) together with those in the present paper, suggest that the lethal effect of the α-toxin in cats arises from cardiac failure, and that the cause of failure might be twofold—an increased peripheral (pulmonary or systemic) vascular resistance induced by intense vasoconstriction, and coronary insufficiency produced by coronary constriction. Kellaway *et al.* (1930) thought that coronary constriction may be the final precipitating factor. The observations of Dingle, Hoff, Nahum & Carey (1937) on rabbits injected with staphylococcal culture filtrates are of interest in this connection, for they found that the electrocardiographic changes preceding death were "in every way comparable to the changes found following occlusion of the coronary arteries." If this view regarding the lethal action of the α-toxin is correct, then the persistence of its effect on the isolated heart appears to rule out the possibility that the lethal action of the toxin might be prevented by any coronary vasodilator agents at present available. So far, only the prior administration of the antitoxin has been found effective in protecting against the lethal, vasoconstrictor or spasmogenic actions of staphylococcal α-toxin.

SUMMARY

1. The effects of staphylococcal α-toxin on the arterial blood pressure and peripheral blood flow in the anaesthetized cat, and on coronary flow and ventricular contraction in isolated perfused cat and rabbit heart (Langendorff) preparations have been studied.

2. Intravenous injection of α-toxin into anaesthetized cats produced a triphasic blood pressure change, consisting of an immediate depressor response, followed by a rise of pressure to near or above the initial value, then a second fall of pressure ending in death. The initial depressor response was accompanied by peripheral vasodilatation, the intermediate pressor and second depressor responses by marked peripheral vasoconstriction.

3. In the isolated heart, the α-toxin greatly reduced coronary outflow. This was followed by depression of the ventricular contraction. The latter effect was considered to be secondary to the change in coronary flow.

4. It was concluded that the peripheral and coronary vasoconstrictor effects of the α-toxin reflect a direct action of the toxin on the vascular smooth muscle. The relation between these effects and the lethal action of the toxin is discussed.

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